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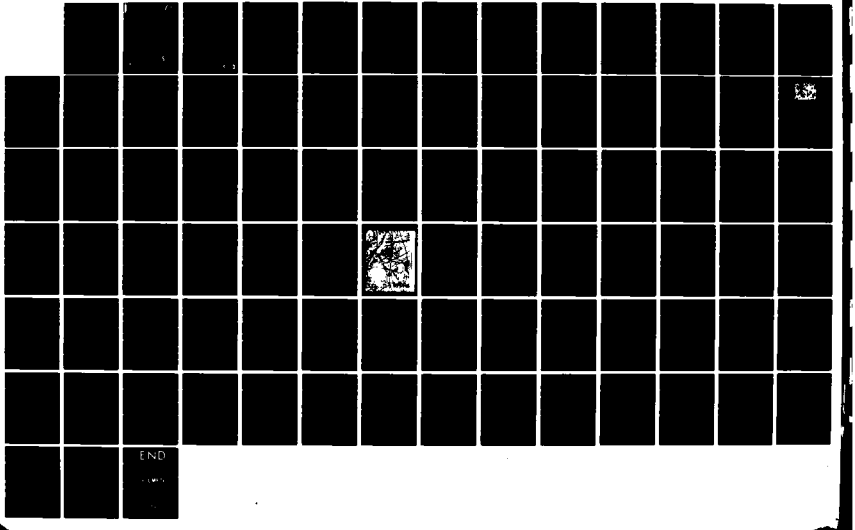

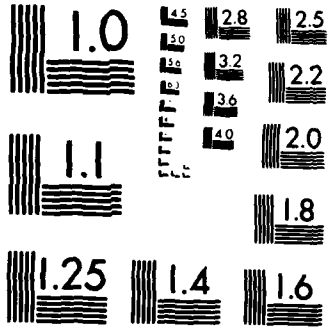

RESEARCH AND DEVELOPMENT OF WOUND DRESSING IN MAXILLOFACIAL TRAUMA

ANNUAL REPORT

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RESEARCH AND DEVELOPMENT OF WOUND DRESSING IN MAXILLOFACIAL TRAUMA

ANNUAL REPORT

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During the contract period three batches of poly-L(-)lactide were prepared and blended with material in stock to give 1,904 grams of polymer with an R.S.V. of 1.14 dl/g. About 1,400 grams remain. Polymer was used to prepare three basic formulations, non-woven fabrics, powders, and microcapsules, of local anesthetic agents, antiseptics, and antibiotics. These formulations were characterized by scanning electron microscopy, in vitro drug release rate, and in vivo efficacy.

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Abstract Continued

Efficacy evaluation of sustained-release anesthetic fabrics was concluded. Using a rat sciatic nerve block model etidocaine fabric was shown to induce good local anesthesia.

Three antiseptics, povidone iodine, (PVP-I_2), nitrofurazone, chlorhexidine diphosphanilate were studied. Povidone iodine microcapsules released drug in a sustained manner for at least 24 hours either as free microcapsules or imbedded in Tegaderm™ or Op-site™. The stability of the drug was studied to provide a guide for preparing efficacious dressings. While the material is stable in vitro significant degradation occurred in serum. In none of the efficacy studies did povidone iodine formulations successfully control the induced infection.

Nitrofurazone fabrics demonstrated sustained release in vitro which could be altered by changing drug-polymer ratio, adding detergent, and by size selection. While nitrofurazone was shown to be bacteriostatic in vitro, neither pure drug nor powder was efficacious in vivo.

Chlorhexidine diphosphanilate was released in a sustained manner by fabrics and powders. Microcapsules were prepared but not tested. Since a suitable microorganism was not available in vivo efficacy tests have yet to be initiated. Of the three antiseptics, povidone iodine and nitrofurazone do not appear to have the required efficacy. This should be confirmed and chlorhexidine diphosphanilate must still be evaluated.

Two antibiotics, ampicillin and clindamycin, have been utilized. Fabrics prepared from ampicillin (trihydrate and anhydrous) provided slow drug release in all test systems. Release rate could be increased by adding sodium dodecylsulfate (S.D.S.), which is both a detergent and increases the solubility of ampicillin, to the diffusion media or incorporating it into the matrix. Fabric prepared from the more soluble sodium salt released drug very rapidly as did all ampicillin powders. Ampicillin microcapsules showed nearly zero order release characteristics. The incorporation of ampicillin trihydrate microcapsules into an ampicillin fabric provided a material with intermediate release characteristics. Ampicillin degradation was a major problem, particularly since the products absorb in the u.v. region and interfered with the drug assay. An H.P.L.C. assay was developed and demonstrated that at room temperature ampicillin was most stable in 40 mM phosphate buffer at pH 6.5. In the in vivo wound model pure ampicillin, 20 mg, did reduce bacterial counts significantly but did not produce a sterile field in 3 days. Ampicillin fabric (5 mg of drug) with and without S.D.S. gave a sterile wound in 4 of 5 animals with 6 days of treatment. Significant amounts of ampicillin were present in the wound and dressing at the end of treatment. However, only in the first day was drug detected in serum (0.12 ug/ul). Ampicillin formulations have demonstrated both the required in vitro characteristics and in vivo efficacy.

Clindamycin fabrics and powders have been prepared and tested. The fabric released drug rapidly in all test systems and release was not reproducible. Similarly, poor results were obtained in the in vitro studies of clindamycin hydrochloride powders. Therefore microcapsules were prepared but they have not yet been tested. In vivo efficacy tests will be initiated when a suitable organism is available.

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In conducting the research described in this report, the investigator(s) adhere to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of (DHEW Publication No. (NIH) 78-23, Revised 1978).

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I. SUMMARY

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II. INTRODUCTION

A. Objectives

The initial objective of this contract was to incorporate antiseptic, anesthetic and hemostatic agents into a single wound dressing, with slow release of the antiseptic and anesthetic agents for optimum effectiveness with the fewest changes of the wound dressing. The polymer used in the drug polymer matrix would be biodegradable (poly-L(-)lactide), allowing fragments of the wound dressing to be left in the wound and absorbed without incident. Initially on this contract, homogeneous drug-polymer formulations of non-woven fabric and powders were prepared and tested in vitro. On a sister contract (DAMD-17-80-C-0110) microcapsules (core/wall particles) of local anesthetics were being prepared and tested, in vitro and in vivo.

In a later modification of the contract microencapsulation of antiseptic drugs was added and hemostatic agents were removed from the scope of work. At a later time local anesthetics were removed from this work scope, and the local anesthetic microcapsule contract was terminated. The last contract modification added antibiotic drugs to the work scope of this contract.

During the present reporting period (March 1, 1983 to June 30, 1984) the contract was renewed in May, 1983 and modified to include antibiotics in January 1984. Work on anesthetics was terminated in May, 1983.

B. Military Relevancy

A combat injury to the maxillofacial area requires immediate attention to stop the bleeding and generate a sterile site for wound healing. The injured soldier may also be required to continue fighting or be able to evacuate the area under his own power. Thus a general analgesic agent such as morphine may not be useful, and a local anesthetic agent would be advantageous.

For less severe injuries, sustained-release drug delivery in military medicine would allow personnel to perform vital combat functions after

Hemostasis is the first task of the soldier or paramedic. Pressure and wound dressing contact are necessary immediately. The more surface area of dressing contact, the faster the bleeding will stop. After the initial blood loss, capillary bleeding may continue. This should be minimized, but blood flow to the site of the injury should not be compromised. Certain materials, such as collagenous protein (e.g. Gelfoam™, Avitene™) offer specific interaction with blood components (platelets) and are excellent hemostatic agents.

Recently, acute pain treatments have been advocated which deliver sufficient analgesic for pain relief without the patient requesting additional drug. This approach eliminates the learned pain response which so often leads to drug dependence following PRN dosing (U. Washington, School of Medicine, 1983). Pain treatment for soft tissue damage is normally scheduled for 2-3 days. For bone involvement the pain treatment extends for a week or two. Slow release anesthetics would aid in this time-contingent management of acute pain.

Infection of the wound site is the most common medical complication after the initial blood loss and shock symptoms have been stabilized. Combat wounds are characterized by a high incidence of infection. This may be due to the presence of devitalized tissue, the presence of foreign bodies in the wound, and/or because there is an unavoidable delay in treating wounds in a combat situation. The immediate treatment may require cleansing the wound area, prior to bandaging. The effectiveness of this procedure depends both on the skill of the paramedic and on the field conditions. The infection process is peculiar to the microorganisms causing the infection, and to the mode of operation of the antibiotics or antiseptics which are used to combat the infection. However, the advantage of the continuous presence of the anti-infective agent at the wound site has been demonstrated for many wound dressings, and also for systemic antibiotic (p.o., i.v., etc.) regimens used to control local infections. Finally, the use of biodegradable polymers in this application is advantageous, since fragments of the wound dressing which are left in the wound will be absorbed without incident.

C. Technical Background

1. Selection of Drugs

a. Antibiotics

The selection of antibiotics was made at USAIDR, with respect to the materials which were being developed by other contractors in their programs on maxillofacial wound dressings. Ampicillin and clindamycin were chosen for this program.

Ampicillin is available from several sources and is available in several physical and chemical forms. Commercially available forms are as the sodium salt (crystal or lyophilized) and as the acid (anhydrous or trihydrate). Bristol Laboratories is the major domestic supplier of ampicillin, and a good source of technical information. Sodium ampicillin and the acid trihydrate were obtained from Bristol Laboratories. A small quantity of anhydrous ampicillin was obtained from Sigma Chemical Company. Lyophilized sodium ampicillin was purchased from H. Reisman Corporation, (Orange, NJ).

Clindamycin is available only from Upjohn and is under patent protection. It is available as the simple hydrochloride or as various esters. For our slow release product we would not require an esterified material. One hundred grams of clindamycin-HCl (Cleocin™ U-21251F Potency 864 ug/mg) was requested and received from the Upjohn Company. The base form of clindamycin is readily prepared by titrating the hydrochloride in water with sodium hydroxide. The crystals are collected on filter paper and washed with small quantities of water.

b. Antiseptics

The use of antiseptics in a wound is of obvious importance. The wound area is localized and the microbial contamination covers the gamut of viable aerobic and anaerobic bacteria, spores, fungi, and yeasts. A high concentration of antiseptic is required for the initial microbial challenge. However, time of contact is also important, and the relative therapeutic effectiveness of inter-

mittent and continuous dosage regimens for antibacterial agents is uncertain (Toothaker, Welling and Craig, 1982). Correlations have been attempted with various pharmocokinetic parameters such as the maximum plasma concentration and the time during which the drug level exceeds the minimum inhibitory concentration. Too high a concentration of an antiseptic may cause tissue cell damage and too short a time period may not allow contact during the life cycle of the bacterial cell at which it is most susceptible to biocidal agents. Thus a time release of antiseptic agents should be useful. Specific studies (e.g. Georgiade and Harris, 1973, for povidone iodine) have shown significant advantages of multiple dosing regimens for antiseptics.

During the present contract period nitrofurazone was later added as an alternate antiseptic. Nitrofurazone (Furacin™, Norwich-Eaton) is used as a local antibacterial agent. It has a wide spectrum of activity and most bacteria of surface infections of the skin or mucosal surfaces are sensitive to the drug. Bacteria develop only a limited resistance to the drug and cross-resistance with sulfonamides and antibiotics does not occur. Nitrofurazone retains its activity in blood, serum and pus. Phagocytosis is not inhibited and it does not interfere with wound healing (Remington's Pharmaceutical Sciences, 14th Ed., p.1184, 1970). For the best effect it should be applied for at least 24 hours; hence a drug release formulation would be advantageous. Nitrofurazone concentrations for bacteriocidal action are approximately 1:100,000. Hence small quantities would be required in a wound dressing. In reading secondary sources such as Goodman and Gilman's "The Pharmacological Basis of Therapeutics" (6th Edition, 978-9), nitrofurazone is compared very favorably with other antiseptics.

Norwich-Eaton Pharmaceuticals sells nitrofurazone as Furacin™ soluble dressings, cream, powder and solutions. For product support they list 56 major human clinical studies. Early work on wound treatment was performed by the military (Snyder, et al, 1945, McCollough, 1947).

Cedergren, et al, (1952) wrote an extensive article and noted the importance of the correct ointment base. Friedgood and Ripstein (1953) noted that sensitive wounds were more easily treated by sprinkling of a powder than by applying ointment or changing dressings. They preferred a powder insufflator to a shaker-top vial.

Zydek (1954) treated 630 wounds, including 287 gash wounds. Results for the gash wounds were listed as very good (148), good (139), questionable (0), and unsuccessful (0).

Gilliotte (1960) also tested a shaker-top vial and preferred a squeeze bottle having a nozzle orifice (insufflator). He used the powder on 256 patients, including 155 traumatic lacerations and 3 other avulsive injuries.

Frenmil and Fishel (1970) compared several nitrofurazone dressing methods in 195 patients with various wounds. Healing was rapid or normal in 93% of the cases. Sensitization occurred with 4 patients and slow healing occured in 9 cases (13-33 days).

Cedergren, et al (1952) also discussed hypersensitivity. Sensitivity is a problem with long term use of the drug and is exacerbated by use of certain vehicles. It is not considered a serious problem by any of these investigators when nitrofurazone is used for traumatic injuries.

Chemically, nitrofurazone is quite insoluble in water. The drug is relatively stable, although sunlight and excessive heat should be avoided. It is less stable than BAC but is more stable than povidone iodine.

Most recently chlorhexidine diphosphanilate was added as a potent antiseptic drug. This is a proprietary product which is available only from Westwood Pharmaceuticals (Bristol Laboratories). It had been made available to the U.S. Army Institute of Surgical Research, and tested by Dr. McManus. We received 100 grams of this material from Westwood Pharmaceuticals for our in vitro and in vivo studies.

Remington's Pharmaceutical Sciences (16th Ed., p.1101, 1980) states that chlorhexidine is bactericidal to both gram-positive and gram-negative bacteria, although it is not as potent against the latter. In a 4% aqueous solution as a surgical scrub, it decreases the cutaneous bacterial population more than either hexachlorophene or povidone iodine. Chlorhexidine is used for the preoperative preparation of both surgeon and patient for the treatment of burns and the irrigation of wounds and surgical infections. Chlorhexidine was obtained as the diacetate from Sigma Chemical Company.

Phosphanilic acid (p-aminobenzenephosphonic acid) is not readily available, nor is there extensive literature on this compound. Thayer, Magnuson and Gravatt (1953) describe the antibacterial action of several compounds, including phosphanilic acid. Kanitkar and Bhide (1947) give some data on the effectiveness of phosphanilic acid against Staphylococcus aureus. Lee, et al (1980) describe the bioavailability and metabolism of phosphanilic acid. In general the activity is similar to the sulfanilamides.

2. Wound Dressing Application Systems

The convenience of application of the wound dressing is important since the dressing should be applied very rapidly after the wound is cleaned. Also a convenient package allows a known dose to be applied to a specific wound area.

The fabric form of a wound dressing offers obvious advantages over a solution or a powder. However the fabric may require a backing as discussed in the earlier section. This backing is presently a gauze bandage material and it serves as a target for spraying and as a form for cutting the fabric to known size. Without backing the material can be readily compressed or stretched. There may be significant advantages to materials such as Avitene mat, or wound dressings such as Op-site™ or Tegaderm™ as the backing material.

For promotion of wound healing some of the materials which are being intensively studied as burn dressings may offer special advantages. USAIDR (Col. Vincent) observations that the wound dries out excessively with time, suggests that a backing should be used which prevents excessive water loss. Suggested materials include Op-site™ (Smith and Nephew, Acme United, Fairfield,

Conn.) and Tegaderm™ (3M, St. Paul, Minn.). Op-site™ and Tegaderm™ are thin polyurethane films with good water vapor and oxygen transmission properties. They are supplied with a contact adhesive of polyvinylether or acrylates.

Powders are more difficult to apply uniformly and reproducibly to an open wound. The powder can be poured into the wound, sprayed from a powder sprayer such as an insufflator, or applied to a wound dressing prior to application to the wound. All three approaches have been considered.

The povidone iodine microcapsules have been sent to USAIDR on Tegaderm™ and in unit dose form in heat-sealed packets. Commercially available blister packs are available which allow delivery to a specific location. An individualized container having a breakable spout on the blister pack is possible (Morton Salt, discontinued).

Application to the bandage is a common method of medicating a wound. Using the acrylate contact adhesive of dressings such as Tegaderm , a layer of powder could be uniformly applied to the wound. This material is available from 3M as Tegaderm™ and as precoated transfer adhesive systems. Multiple layers of microcapsules should be attachable using adhesive approaches. Very thick layers of microcapsules would be more readily achieved by filling a spacer grid placed between two layers of gauze which would act as a sieve to prevent the loss of drug particles. This is a practical problem which can be solved at a later time.

III. ACCOMPLISHMENTS

A. Polymer Synthesis

Polylactide had been prepared several years ago for this contract and for the anesthetic microcapsule contract (DAMD17-81-C-1195). This preparation was described in the Annual Summary Report dated June 23, 1982, and was quite time consuming.

When the supply of polymer which had been prepared for Contracts DAMD17-81-C-1204 and DAMD17-81-C-1195 had been exhausted, a new batch of polymer was prepared for use only on Contract DAMD17-81-C-1204. Lactide dimer from Boehringer-Ingelheim was recrystallized from ethyl acetate. The goal was to produce three batches of 500 grams each, which had reduced specific viscosities (R.S.V.) of 1.2 to 1.8 dl/g. This could be blended with polymer in stock, which had an R.S.V. of 0.70 dl/g, to give a blend having an R.S.V. of 1.2 $\pm$ 0.1 dl/g.

For the first polymerization batch, the dimer was recrystallized twice. The polymer product was dissolved in methylene chloride and a small sample was reprecipitated using isopropanol. The R.S.V. of this material was 1.69 dl/g. For the next polymerization the dimer had to be recrystallized three times, and the R.S.V. of the product was 1.53 dl/g. In the final batch the dimer was recrystallized three times and the R.S.V. was 1.76 dl/g.

These three batches had a combined weight of 1506 grams and was blended with 1000 grams of 0.70 dl/g polymer. This material was precipitated with isopropanol and the final blend had an R.S.V. of 1.14 dl/g. The product was dried in the air suspension chamber, and 1904 grams of polymer was recovered. Thus the total yield was 76%. This apparently lower yield is probably due to better drying of the polymer. The previous polymer was not dried in the air suspension chamber, and a later GC analysis of the polymer indicated 15% isopropanol. The present polymer is dry, and has been sealed under argon in metal cans. It is now being stored in the freezer. As of June 1, 1984, 1400 grams remain to be used.

B. Development of Polymer/Drug Materials

1. Non-Woven Fabric Preparation

The polymer-drug fabric is prepared as in the last annual report dated March 13, 1983. About a 10% (weight-to-volume) solution of the polymer is prepard using methylene chloride as the solvent. The appropriate amount of drug is added to this system to obtain the appropriate drug/polymer ratio (e.g. 20%). This solution is poured into a tank which is connected to the liquid inlet of air atomizing nozzle using a piece of tubing.

The air inlet is connected to a source of compressed gas. Fine polymer-drug fibers are formed as the solution leaves the nozzle. The fibers are collected on a piece of surgical gauze. This material is held at a fixed distance from the gun nozzle and is attached to a frame to keep it taut. Thus air passes through the wound dressing to facilitate drying of the solvent.

2. Powder Preparation

The second form of the BIOTEK wound dressings is particulate. In this form combinations of powders can be added as required. Separate powder particles can be hemostatic or contain antiseptic or anesthetic agents. Separate particles can be tailored for optimal delivery of a specific drug. Microencapsulation of povidone iodine has been shown to yield the best continuous *in vitro* release of active iodine. These oxidizing particles can probably be added to particles of less stable anesthetics such as the base form of lidocaine, provided the system is kept dry. A non-woven fabric containing both of these materials has been demonstrated to be unstable, under ambient room conditions.

Powders have been made by by comminution of a homogeneous film of polylactide and drug. In this approach the drug release is expected to be non-linear. The mathematics generally follow the Higuchi equation ($Q = kt^{1/2}$). The rate of release decreases with time as the surface layers of the particle or fiber become depleted of the drug. Baker and Lonsdale (1974) have published the equations for these systems. Since more drug may be needed initially, this approach of using homogeneous drug/polymer materials is appropriate for wound

dressings. However, in many cases, drugs are not released from the center of the particle or fiber within a reasonable time period. This is especially true of high molecular weight materials such as povidone iodine.

During this report period a hammer mill was purchased and assembled at BIOTEK. This was used for the preparation of powders on this contract. Solutions or suspensions of various drugs in a 10-15% polymer solution are cast on glass plates.

A Mikro-Pulverizer C.F. (18,000 RPM, Pulverizing Machinery, Div. Slick Industrial Co., summit, N.J.) is used for grinding these films. The mill is assembled with the hammer edge of the rotor facing the direction of rotation. A 20 mesh (841 um) classifier screen was used at the bottom of the mill. The feed and comminution chamber were pre-cooled with 500 grams of dry ice. Next the sample was sent through the mill, mixed 1 to 10 with dry ice (i.e., 90% cardon dioxide). A final charge of 100 grams of dry ice was sent through the mill to increase the yield from the chamber. The mill was dismantled, washed, dried, and reassembled for each drug run.

3. Microcapsule Preparation

The second method of making wound dressing particles is by microencapsulation. In this approach a particle of drug is coated with a layer of polymer. A continuous constant release is possible with this approach, since a constant concentration gradient can be maintained across the wall of the microcapsule. In practice some non-linearity is observed in the rate of release, because the microcapsules are of varying sizes and have imperfections in the capsule walls. However, drug release is more continuous than for homogeneous systems, and can approach a constant rate in some cases (e.g. Nuwayser, et al, 1976).

Under Contract DAMD17-81-C-1195, entitled Local Anesthetic Microcapsules, lidocaine, etidocaine and bupivacaine have been microcapsulated with polylactide, using the Wurster process. This work has generated microcapsules having a wide range of in vitro release rates. The rates are dependent on the choice of drug, the amount of polymer coating and the size range of the microcapsules. Slow release has been demonstrated in vivo by measurement of circulating blood

levels of the anesthetics in rabbits. Lower systemic toxicity (4-7 times less toxic than the soluble drug) has been shown by LD50 and convulsive dose studies in mice. Lower tissue toxicity of microencapsulated lidocaine has been demonstrated by CPK analysis after injections of solutions and microcapsule suspensions in rabbits. The duration of anesthesia was measured by the blockage of the sciatic nerve of the rat using etidocaine-HCl microcapsules (150-212 um, 47% drug content, 50% *in vitro* release in 23 hours). Most blocks which lasted 12 hours also lasted 48 hours. The median effective dose for this anesthesia (ED50) was approximately 50 mg (150 mg/kg). The convulsive dose (CD50) was 330 mg/kg in this experiment. With etidocaine solutions 48 hours of anesthesia could not be achieved. One day blockage was achieved, but the dose was above the convulsive dose (ED50 > CD 50).

The contract on wound dressings has benefitted from the effort on microencapsulated anesthetics. The method of encapsulation is the same as for the microencapsulated anesthetics.

C. *In Vitro* Drug Release Studies

The measurement of drug release as a function of time is the primary measurement of this program. The standard method of analysis is to measure drug release into a large (40 ml) volume of phosphate buffer. In this procedure the sample is maintained at 37°C and agitated. In a secondary procedure, drug release is measured into a small (1 ml) volume of solution (buffer, serum, etc.) which is maintained at room temperature (21° $\pm$ 3°C) without agitation. The preferred method of drug analysis is by spectrophotometry. Iodine was initially measured by thiosulfate titration, and later by the absorbance of the starch-iodine adduct. In a complex system (e.g., ampicillin release into serum), liquid chromatography (HPLC) is used with a spectrophotometric detector for the drug analysis.

Drug release rates are determined by suspending a known quantity of drug in a powder, microcapsule, or fabric form in a known volume of an aqueous solution and periodically measuring the concentration of the drug in the solution. From

the solution volume and concentration, the quantity of drug released is computed. This quantity divided by the length of time since the last measurement is the average drug release rate over the time interval.

In the standard 40 ml test system the suspending solution is water buffered with phosphate (pH 7.4 Sorenson's buffer, 0.04 molar). These solutions are thermostatted at 37°C in a metabolic shaker bath. The vessel used for the release studies is a specially designed L-shaped test tube. The shape of this container promotes good mixing of the release solution when used in a metabolic shaker and thereby reduces local drug concentration gradients in the solution which might affect the release rate. The polymer-drug composites are placed in a tea-bag type structure, constructed from fine polyester mesh for convenience in separating the fabrics and particles from the suspending solution.

During this report period, BIOTEK installed a small computer facility and the in vitro diffusion data of this program has been entered on computer disk for ease of entry, storage, sorting, and graphing. We are using a LOTUS 1-2-3 worksheet program which is available for our IBM Personal Computer. This package also includes a GRAPH-program disk.

1. Antiseptic Materials

a. Povidone Iodine Microcapsules

Microcapsules of povidone iodine were prepared during the previous report period. During this period a 212-600 um sieve fraction of the 30% coated core was submitted for in vivo studies and stored for stability studies. A small sample was submitted on Tegaderm™.

The microcapsules of povidone iodine (11-5-30, 212-600) adhered well to the contact adhesive used in Tegaderm™ and Op-Site™. Approximately 20 mg of microcapsules could be imbedded per square centimeter of surface area of either bandage. Slightly more microcapsules could be imbedded if a rolling pressure was applied to the microcapsules between successive applications of microcapsules. This quantity of microcapsules approximates a monolayer of capsules. Microscopic examination showed an even distribution of microcapsules over the adhesive surface. Drug release studies were run with microcapsules imbedded in Tegaderm™ and in Op-Site™. These data are shown in Figure 1 and compared with the release of freely dispersed microcapsules. Figure 2 shows an optical photomicrograph of this material.

b. Povidone Iodine Decomposition

In vitro experiments with povidone iodine have been modified to minimize the effect of iodine loss. However there would be advantages to monitoring both the iodine and iodide in solution as povidone iodine is being released from fabrics, powders, and microcapsules. Therefore an iodide and a redox electrode were purchased from Orion Research, Inc., of Cambridge, Mass.

In a preliminary experiment, povidone iodine was titrated with sodium thiosulfate in water and the redox electrode potentials were compared to the visual starch end-point. A typical potentiometric titration curve was obtained. The visual end-point would be chosen at 304 mv; whereas the inflection point of the potential titrant curve was around 270 mv (7.8 vs. 8.0 ml of titrant). Tests with povidone iodine in serum were inconclusive when we titrated a povidone iodine solution with human serum. There was an indication of a slow rate of reaction between povidone iodine and serum proteins.

A series of experiments were then performed to determine the length of time that the active iodine would be present in serum samples, based on the original concentration of povidone iodine in rabbit serum. This information would be valuable in determining the concentration of povidone iodine to be used in a wound to maintain bacteriostatic effectiveness for a given period of time,

Figure 1 In Vitro Release of PVP-I_2 from Microcapsules (11-5-30)

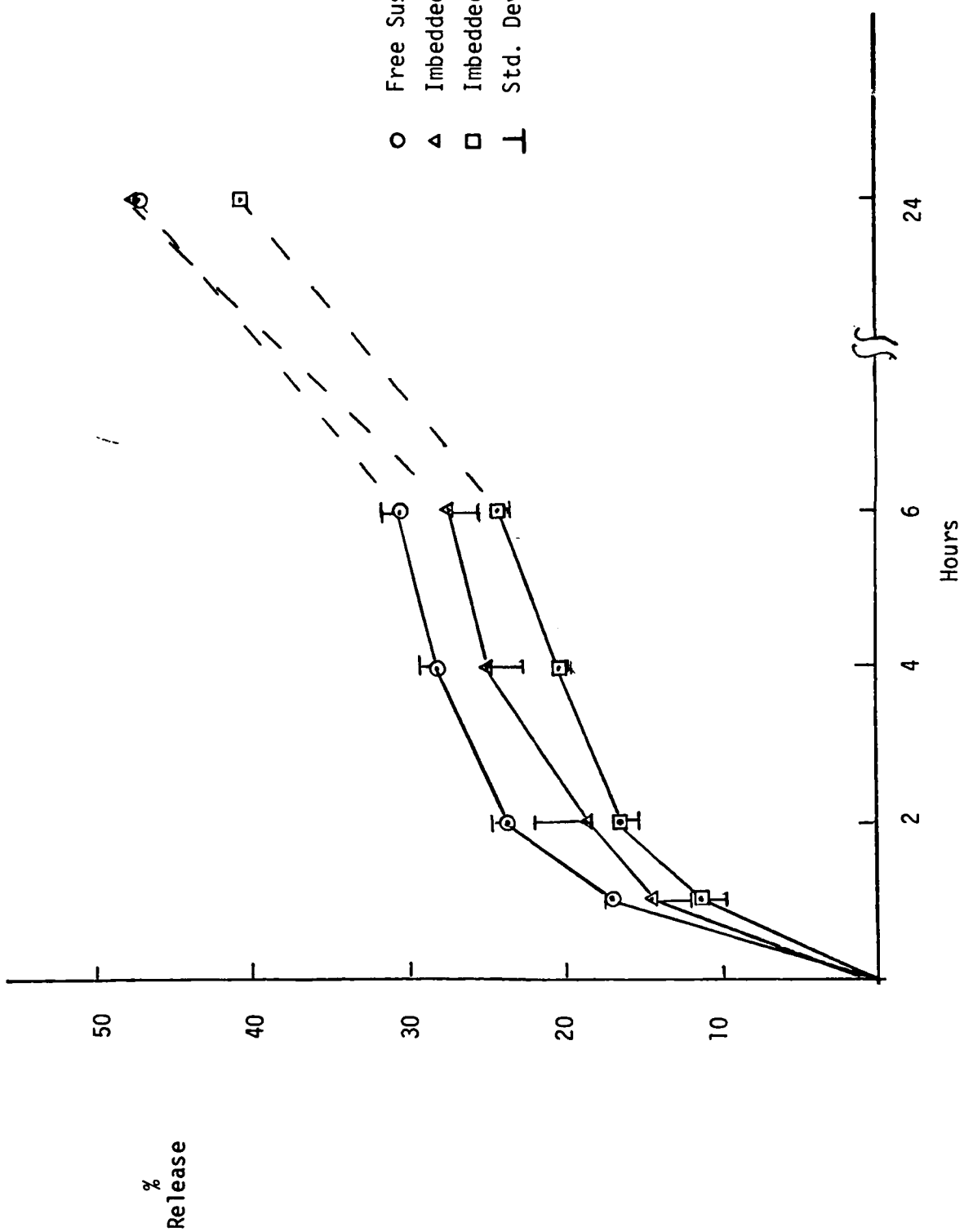
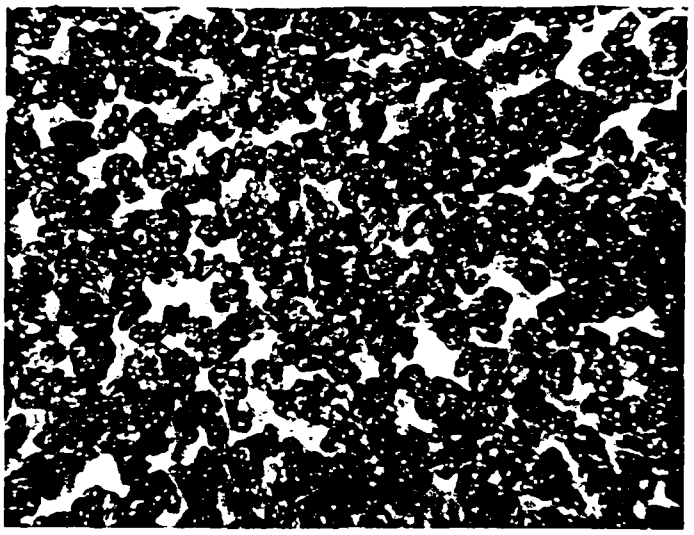


FIGURE 2



Povidone Iodine 70% In Microcapsules
(30% Poly-L(-)lactide Coating)
On Commercial Adhesive Wound Dressing

assuming the povidone iodine was applied as a soluble dressing. It might then be possible to calculate the rate of release needed to maintain this active iodine potential when using a slow release formulation.

Potential time curves were obtained for various concentrations of povidone iodine in serum and the time increased as the initial povidone iodine concentration increased.

If one considers the reaction of iodine with a serum component (e.g., sulfhydryl groups) in which the rate is first order with respect to the remaining serum reducing groups, then:

$$\frac{d[I]}{dt} = -k_1[b-(a-[I])] \quad \text{and}$$

$$\int_{I=a}^{I=0} \frac{d[I]}{b - a + [I]} = -k_1 \int_{t=0}^{t=\tau} t$$

where $\underline{a}$ is the initial povidone iodine concentration, $\underline{b}$ is the initial concentration of serum reducing groups, and τ is the end point as the concentration of active iodine approaches zero ($b > a$).

This equation integrates to

$$\ln \frac{b-a}{b} = -k_1 \tau$$

One can then calculate values for this rate constant $\underline{k_1}$ for various values of $\underline{b}$ and choose the solution which is most consistant ($\underline{k_1}$ is a constant). There is also some effect on this calculation by choosing different redox potentials for

the end point. By choosing 190 mv for the end point the most appropriate value for b is 22 (mg as povidone iodine). This is equivalent to about 0.017 meq/ml of reducing groups in serum, based on an equivalent iodine content of 10% for BASF 17/12 povidone iodine. The rate constant is 0.0068 $\pm$ 0.0010 minutes^{-1} based on the four data points (See Table 1).

Although this analysis gives the sought logarithmic relationship and a constant apparent rate constant, we could find no reference to this approach in standard texts. A second order dependence is probable for this experiment and the appropriate equation is

$$\frac{1}{a - b} \ln \frac{b\ (a - x)}{a\ (b - x)} = k_2 t ,$$

assuming the same end point conditions.

$$\frac{1}{a - b} = k_2 \tau$$

Again we can find b by reiteration, such that k_2 is constant. This reaction hypothesis fails at low iodine concentrations (a), but gives a second order rate constant (k_2) of 1.3 min^{-1} meq^{-1} and a serum reducing group concentration of 0.020 meq/ml (See Table 1).

These analyses may be useful in determining the quantity of povidone iodine required to maintain an oxidizing environment in the wound area for various periods of time. However the contract efforts were then directed towards antimicrobials which have higher activity and stability in the presence of serum components.

TABLE 1

Calculation of Reducing Groups in Serum and Reaction Rate Constants With $PVP.I_2$

$\underline{a}$ mg/ml $PVP-I_2$	$\underline{\tau}$ minutes	if $\underline{b}$ = 22 then k_1	if $\underline{b}$ = 25 then k_2
20	335	0.0072	0.00050
15	187	0.0061	0.00053
10	103	0.0059	0.00065
5	32	0.0081	0.00156
average		0.00682	0.00084
± S.D.		± 0.00102	± 0.00048

c. Nitrofurazone Materials

Nitrofurazone was graciously supplied to us by Norwich-Eaton Pharmaceuticals.

Nitrofurazone is practically insoluble in methylene chloride and no combination of methanol and methylene chloride was found which could dissolve both the drug and polymer. Nitrofurazone was received as a very fine powder and it was readily suspended in methylene chloride. Therefore both fabrics and powders were prepared with a suspension of drug in this polymer solution. Nitrofurazone is measured spectrophotmetrically at 260 nm in buffer, and at 385 nm in dimethylformamide for the assay values of the composites.

1) Fabric

Nitrofurazone fabrics were prepared by the conventional technique at 20% drug loading. Good cohesive non-woven fabric was collected. Drug release was slow as shown in Figure 3. Although nitrofurazone dissolves slowly in water, 90% dissolved in one hour when the pure drug powder was placed in a diffusion cell.

Although the results of drug release from this fabric were variable, we proceeded to make additional fabrics at 15, 30, 40, and 50% nitrofurazone loadings. We expected that the fabrics with more drug (per polymer weight) would release their drug more rapidly. Fabric was formed, even at 50% drug loading, but the drug release was variable at all drug loadings (Figure 4). Incomplete wetting of the sample may be the reason for this variation. A wetting problem had been shown previously with povidone iodine fabrics. Wound exudate or serum may or may not wet these fabrics better than aqueous buffers.

Fabric samples were placed on clean glass slides which have wells bounded by 1 mm high ceramic rings of 1.2 cm diameter (Fisher Scientific, 12-568-30). Various solutions (0.15 ml) were carefully added with a syringe and wetting was observed visually. Solutions of water, buffer, rabbit serum, 0.2% sodium lauryl sulfate and 0.005% alkyl amine hydrochloride (Hyamine™ 2389) were tested. Only the sodium lauryl sulfate wetted the fabric. A sample of 40%

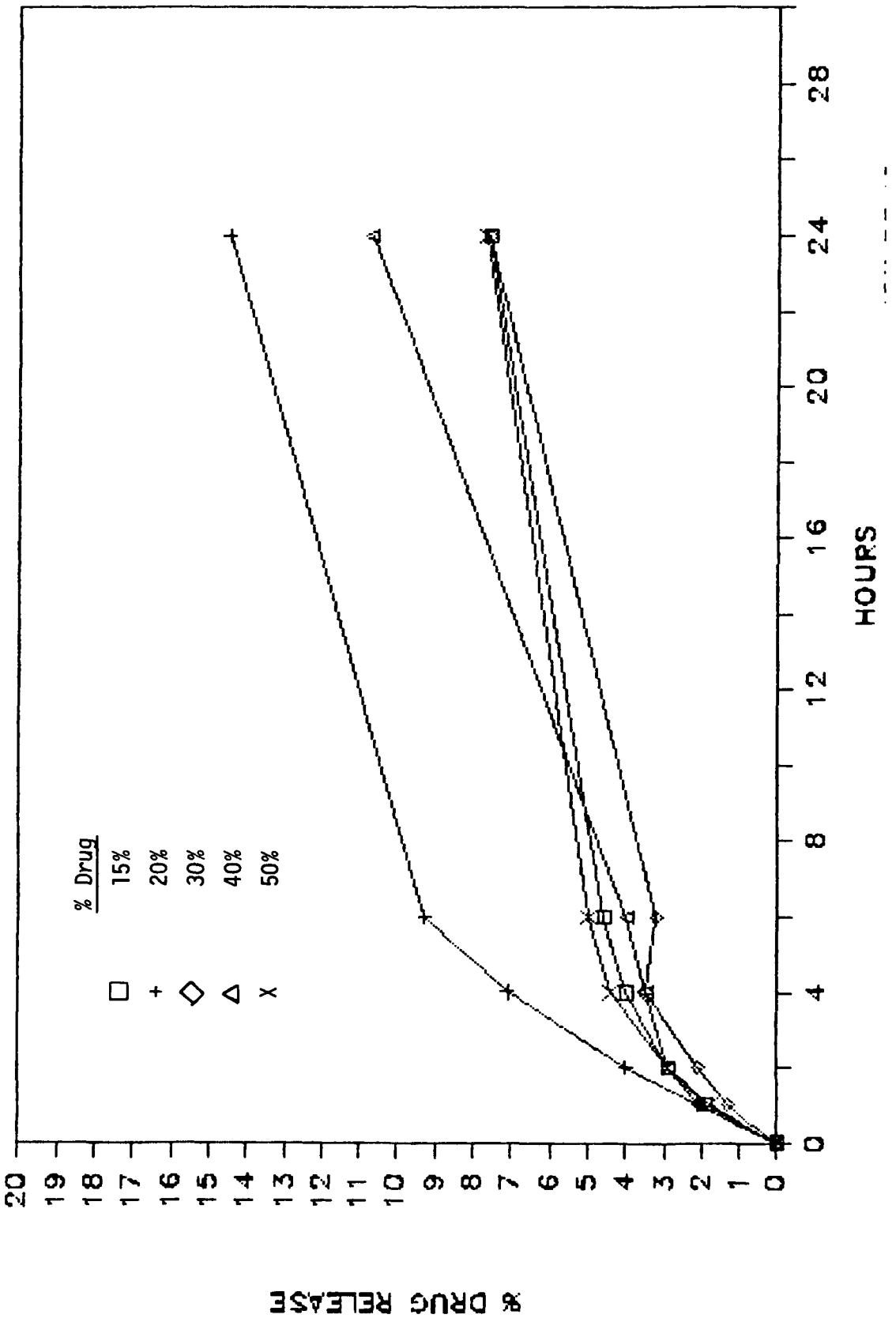
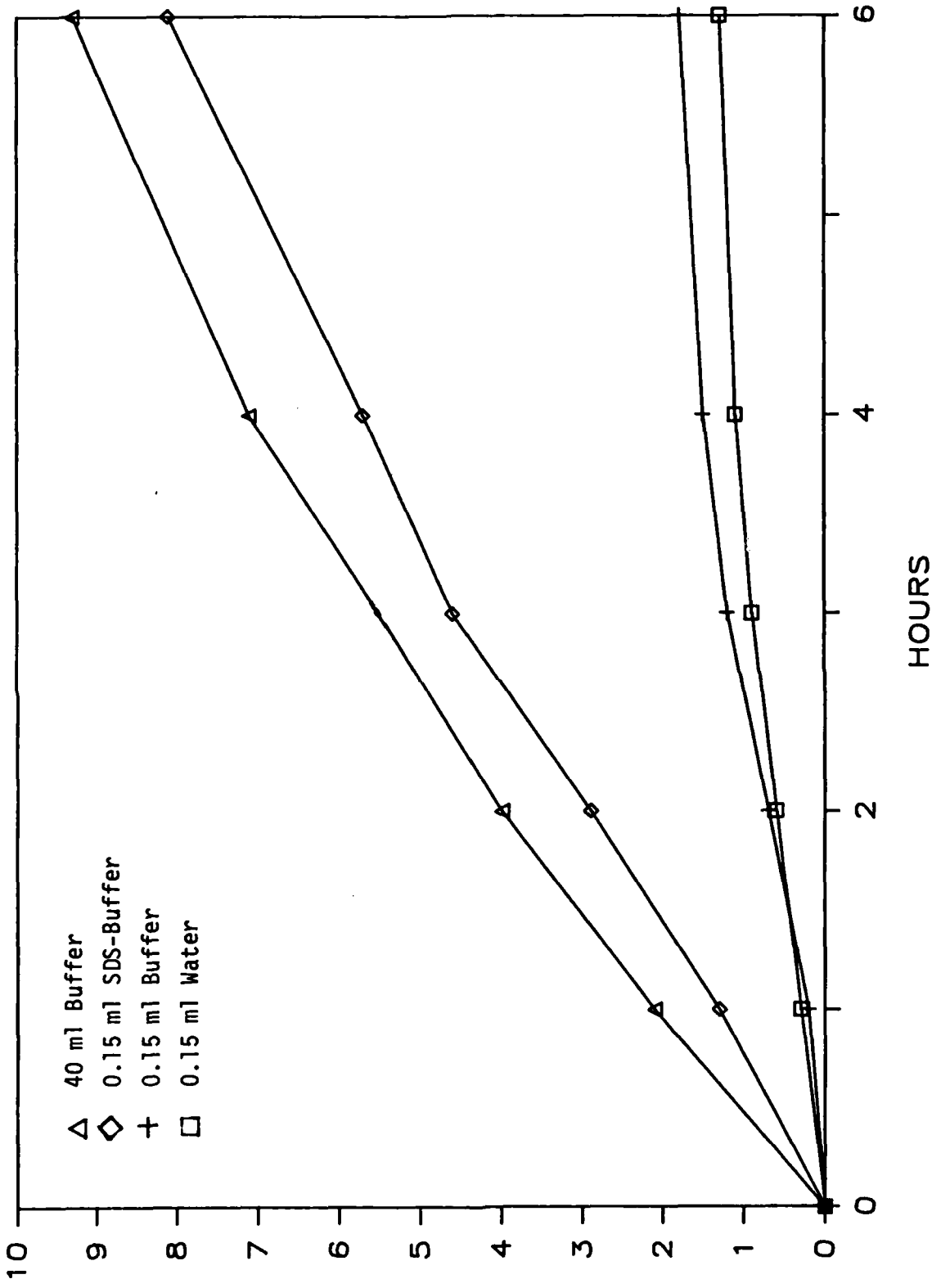


Figure 3 AVERAGE IN VITRO NITROFURAZONE RELEASE FROM FABRICS

FIGURE 4

RELEASE FROM 20% NITROFURAZONE FABRIC



povidone iodine fabric was wetted even with pure water by this test method. Nitrofurazone release was studied into these small reservoirs using water, buffer, and 0.014% sodium lauryl sulfate (SLS) as the wetting and drug release media. Fabric samples (approximately 2 mg) of 20% nitrofurazone were placed in 150 ul of the solution. At hourly intervals 100 ul of the solution was collected for analysis. The sample was then transferred to a new test well and rewetted with 150 ul of the test solution.

Fabrics in the SLS reservoir released 4 to 5 times more drug per hour than fabrics in the water or buffer reservoirs. However reproducibility was not improved. The amount of nitrofurazone released into these SLS wells was comparable to that released by the larger swatches of fabric (13 mg) into 40 ml of 37°C buffer in a shaking system in which the fabric is enclosed in a polyester mesh and submerged in the buffer solution. The data are presented in Table 2.

In addition to demonstrating the wetting problem, this type of test is also more representative of the use of a wound dressing. The fabric must be wetted by the tissue fluid in a static environment with a small amount of downward external pressure which is applied by the bandage. Wetting of the fabric or powder in this environment may be a significant problem. Secondly, concentration gradients within the fabric or powder mass may lower the drug release rates from that observed in a shaking system. This is especially important with drugs which could saturate the local, absorbed solution.

2) Powder

Suspensions of nitrofurazone in polymer solutions in methylene chloride were prepared and cast on glass plates. The drug was suspended in viscous 20% polymer solutions, and there appeared to be very little settling prior to solvent evaporation. These films were broken into pieces which could be fed through the hammer mill with dry ice. The mill was assembled with a 20 mesh classifier screen. The machine was pre-cooled with about 500 grams of dry ice and then the sample (approximately 25 grams) was sent through with 10:1 dry ice:drug-polymer. A final 100 grams of dry ice was sent through the equipment to increase the product yield (decrease hold-up).

TABLE 2

CUMULATIVE DRUG RELEASE FROM 20%

NITROFURAZONE FABRIC

A. Release into 150 µl Reservoir (static, 22°C)

Solution	n	Value	% Release in Specified Hours 1	2	3	4	6
Water	2	Mean	0.3	0.6	0.9	1.1	1.3
		Range	0.0	0.0	0.2	0.6	0.8
Buffer	2	Mean	0.25	0.75	1.2	1.5	1.8
		Range	0.10	0.10	0.3	0.4	0.6
SLS	2	Mean	1.3	2.9	4.6	5.7	8.1
		Range	0.4	1.3	2.0	2.8	5.1

B. Release into 40 ml Reservoir (shaking, submerged, 37°C)

Buffer	3	Mean	2.1	4.0	7.2	-	9.3
		S.D.	0.4	1.1	2.2	-	3.1

Films of 10 and 40% nitrofurazone in polylactide were prepared and ground. Unfortunately a 20% sample was contaminated and therefore was discarded. The size distribution of the samples is shown in Table 3.

Selected sizes of 10 and 40% nitrofurazone powders were analyzed for their drug release characteristics in in vitro tests. As expected, the results (Figure 5) show significant decreases in the rate of drug diffusion with increasing particle size. Smaller rate differences were observed for the same size particle at different drug loadings. Large variations in the rate of drug release can thus be achieved with these powders, and the 300-425 um sieve fraction was sent to USAIDR for animal studies. A sample has also been set aside for stability studies.

3) Microcapsules

Since the nitrofurazone powder at 40% loading delivered the drug at the target rate (i.e. 50% release in approximately 3 hours) and could be varied by a change of particle size, microencapsulation of this drug did not appear to be warranted. A higher drug loading might be possible with microcapsules, but the maximum loading of the powder might also be increased.

d. Chlorhexidine Diphosphanilate Materials

Chlorhexidine diphosphanilate was obtained as a courtesy sample from Westwood Pharmaceuticals (Bristol-Myers).

The uv spectrum of chlorhexidine diphosphanilate was compared with that of a sample of chlorhexidine diacetate and it was determined that both chlorhexidine and phosphanilate absorb strongly at 240 nm. However there was sufficient overlap to make the measurement at two wavelengths and a calculation of both the chlorhexidine and phosphanilate ions impractical.

Chlorhexidine is a strong complexer and precipitates in phosphate buffer, in rabbit serum and in the presence of dodecysulfate ions. At the present time drug release has been measured into pure water and into a dilute quaternary

Table 3

SIZE DISTRIBUTION OF NITROFURAZONE POWDERS

(Data is % of Total Milled Sample)

	Drug in Powder	
Size (um)	10%	40%
600	9.6	2.4
600-425	13.8	4.1
425-300	20.5	10.3
300-212	19.4	19.9
212-150	14.4	20.9
150-106	12.3	21.1
106-74	6.1	11.4
74-38	3.2	7.7
. 38	0.0	0.0
Percent Yield of Milling	81	81

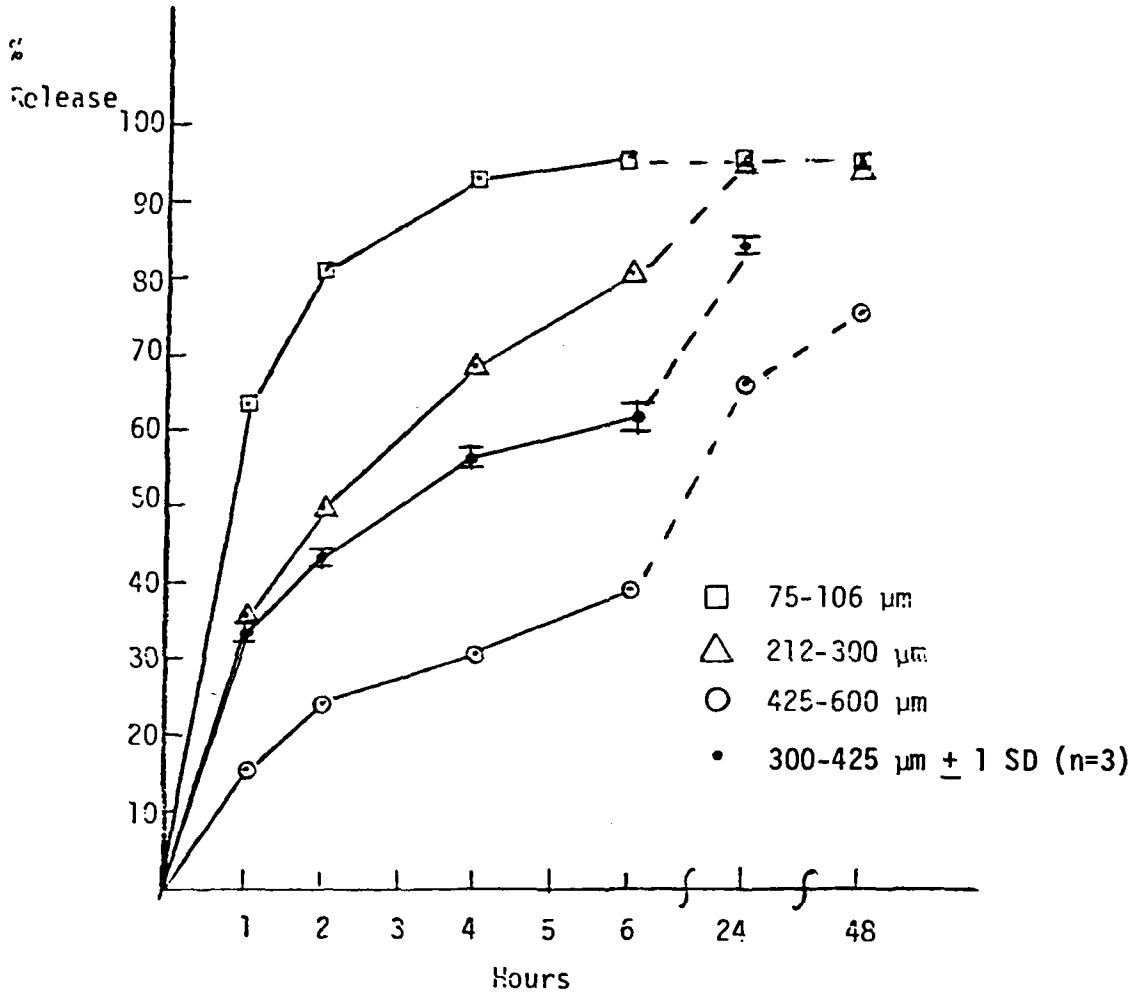


Figure 5 IN VITRO RELEASE OF NITROFURAZONE FROM POWDERS OF 40% DRUG

ammonium surfactant solution (Hyamine™). In these media chlorhexidine and phosphanilate ions are released at the same rate to preserve electroneutrality. An HPLC method is under development to measure the cation and anion independently and to make this measurement in more complex media (e.g., serum).

Chlorhexidine diphosphanilate did not dissolve in methylene chloride, but the suspension in a viscous polymer solution was used to make the fabric and powder.

1) Fabric

A thick fibrous mat of 20% chlorhexidine diphosphanilate was sprayed without incident. Two samples of this fabric were used in the standard (40 ml) diffusion cell with water. Although reproducibility was poor, there was continuous release (see Figure 6, 4% in 1 hour, 15% in 6 hours, and 40% in one day). When using Hyamine the results were remarkably similar (5% in 1 hour, 12% in 6 hours, and 30% in one day). When using a small cell without agitation (1 ml), very little drug was released into either water or Hyamine (4% in 1 day).

2) Powder

Chlorhexidine diphosphanilate was cast at 5 and 20% loading into films and then milled into powders. Only the 20% powder demonstrated continuous release into 40 ml of water. The release was dependent on the particle size, as shown in Figure 7.

The 20% chlorhexidine diphosphanilate powder of 212-300 um sieve size was also tested in the 1 ml cell. The drug release rate significantly slower in this test cell (12% release in 6 hours).

3) Microcapsules

Microcapsules of chlorhexidine diphosphanilate have been recently prepared using the small coating chamber (2 inch diameter). Approximately 11 grams of

FIGURE 6

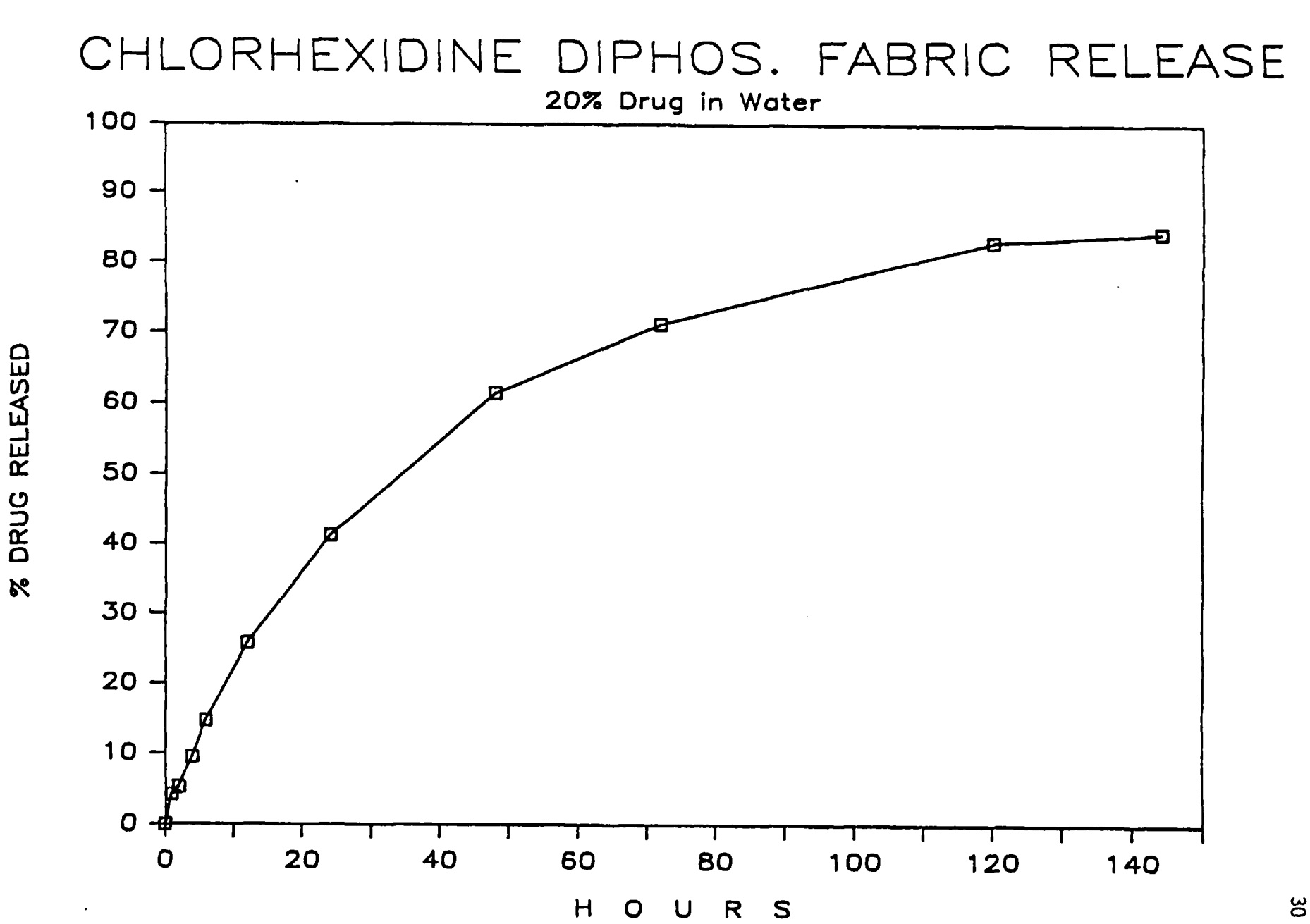
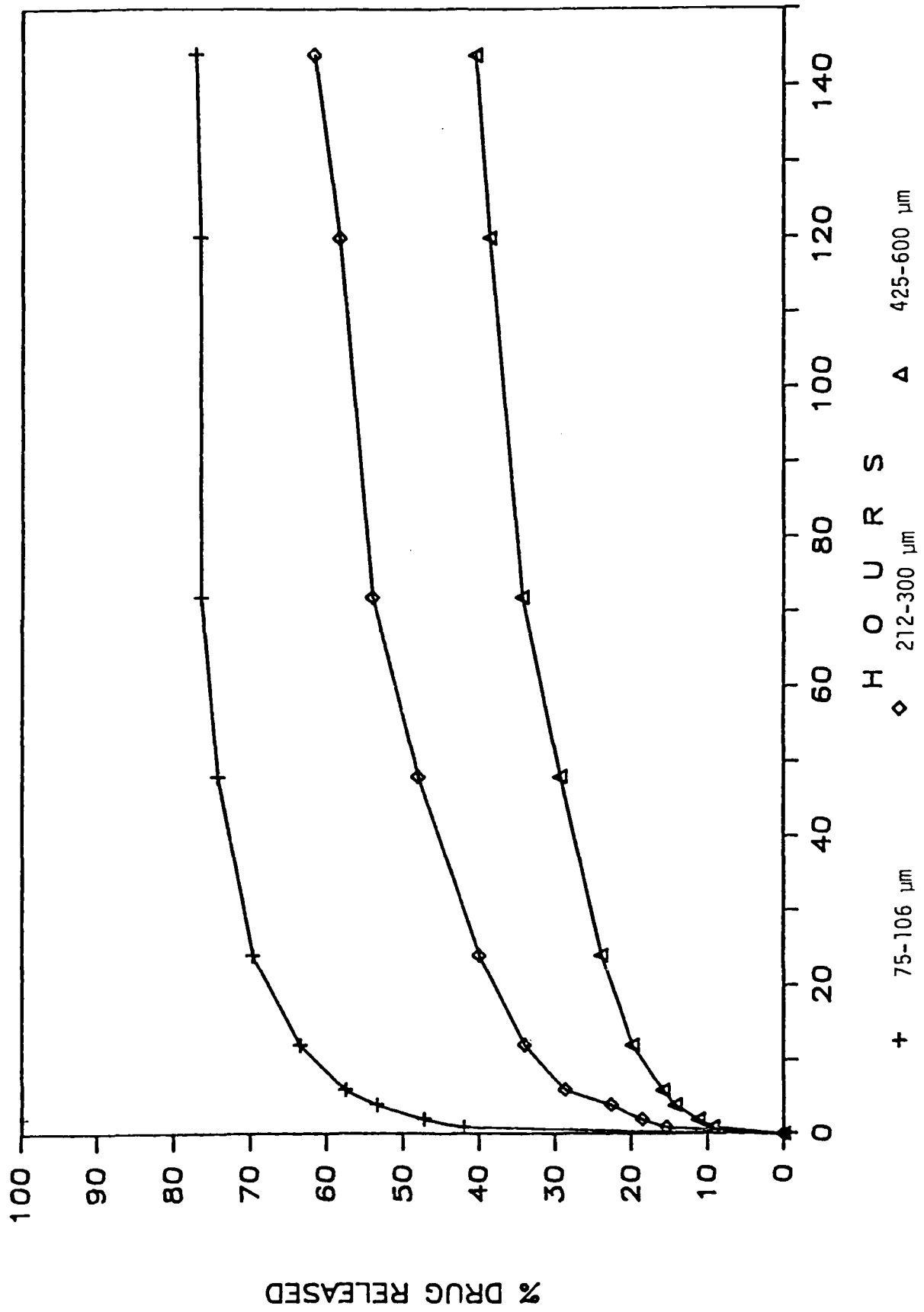

CHLORHEXIDINE DIPHOS. FABRIC RELEASE
20% Drug in Water
% DRUG RELEASED
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
H O U R S

FIGURE 7

CHLORHEXIDINE DIPHOSPHANILATE POWDER RELEASE

INTO 40 ml OF WATER (20% DRUG)



core material was coated to approximately 33% using a 2% polylactide solution. A nominal coating of 33% was obtained and a small sample was taken at about 14% coating.

These samples have not been tested for drug release.

2. Antibiotic Drug Release

Antibiotics are considerably more labile than the anesthetics and antiseptics which have been tested on this contract. Also they tend to be less soluble in organic solvents and less absorbant in the near ultraviolet range of the spectrum.

a. Ampicillin Materials

Ampicillin absorbs more strongly in the uv than does clindamycin, although neither compound shows a uv maximum in the usual instrumental range. At 240 nm ampicillin absorbs strongly enough to be used for in vitro drug release and assay studies (392 ug/ml-AU for the sodium salt, 406 ug/ml-AU for the trihydrate and 324 ug/ml-AU for anhydrous ampicillin in pH 7.4 buffer). The purity of the anhydrous ampicillin is also questionable based on an HPLC peak for this material which was not present in the ampicillin trihydrate sample.

1) Ampicillin Fabrics

Ampicillin comes in variety of forms from several manufacturers. The trihydrate, anhydrous material, and the sodium salts are all insoluble in methylene chloride. However, the trihydrate (Bristol) and anhydrous material (Sigma) are small particles which are readily suspended in methylene chloride. Fibrous products are formed by spraying. Sodium ampicillin (Bristol) is a larger crystalline substance which does not suspend nor spray well. The product was a thin powdery mat. A lyophilized sodium ampicillin has been ordered from H. Reisman Corporation.

a) Ampicillin Trihydrate Fabrics

Initial drug release measurements were made with 20% drug fabric into 40 ml of 37°C buffer solution. Ampicillin trihydrate released only about 2% in the first hour and 4% in the first day or week (Figure 8). However addition of 0.001% sodium dodecylsulfate (SDS) to the buffer increased the rate of release to 15% in the first hour and 79% in one day. Ampicillin decomposition is significant and compromises the release data at longer time periods. Incorporating the SDS in the fabric also increases the rate of release of ampicillin from a 20% ampicillin trihydrate fabric. Addition of 1% SDS in the fabric gave an almost immediate release of ampicillin (87% in one hour). Since the fabric weight was 32 mg, the amount of SDS was 0.32 mg. If this diffused into 40 ml, it would give a maximum solution concentration of only 0.0008%.

These tests were then repeated in the small reservoir cell, without agitation and at room temperature (Figure 9). In these tests about 13 mg of 20% ampicillin trihydrate fabric was placed in contact with 1 ml of buffer, SDS, or serum. Without SDS in the fabric or buffer the drug release was again very slow (1% in one hour and 8% in one day). Release of ampicillin into 0.001% SDS was faster (1% in one hour but 17% in one day). Again the addition of 1% SDS to the fabric greatly increased the rate of drug release (57% in one hour and 84% in one day).

The most important aspect of drug release would be the release into a wound exudate. Serum (rabbit) was chosen as the most practical simulation of this medium for preliminary tests. Calf serum which is a pooled normal mycoplasma-tested virus-screened material has recently been obtained from GIBCO for future experiments. BIOTEK recently obtained an HPLC with ultraviolet detection capability to 214 nm. This instrumentation allows antibiotic measurement in complex systems such as serum. Appropriate methods were found for ampicillin (Tanigawara, et al, 1982) and for clindamycin (Landis, Grant, and Nelson, 1980) using HPLC with a C-18 column and uv detector.

For ampicillin a methanol/water system was suggested and, therefore, we decided to deproteinize the serum with methanol (Henry, Connor, and Winkelman, 1974). The precipitate of 8 volumes of methanol and 1 volume of

FIGURE 8

AMPICILLIN TRIHYDRATE FABRIC RELEASE

20% Drug in 40 ml. Reservoir

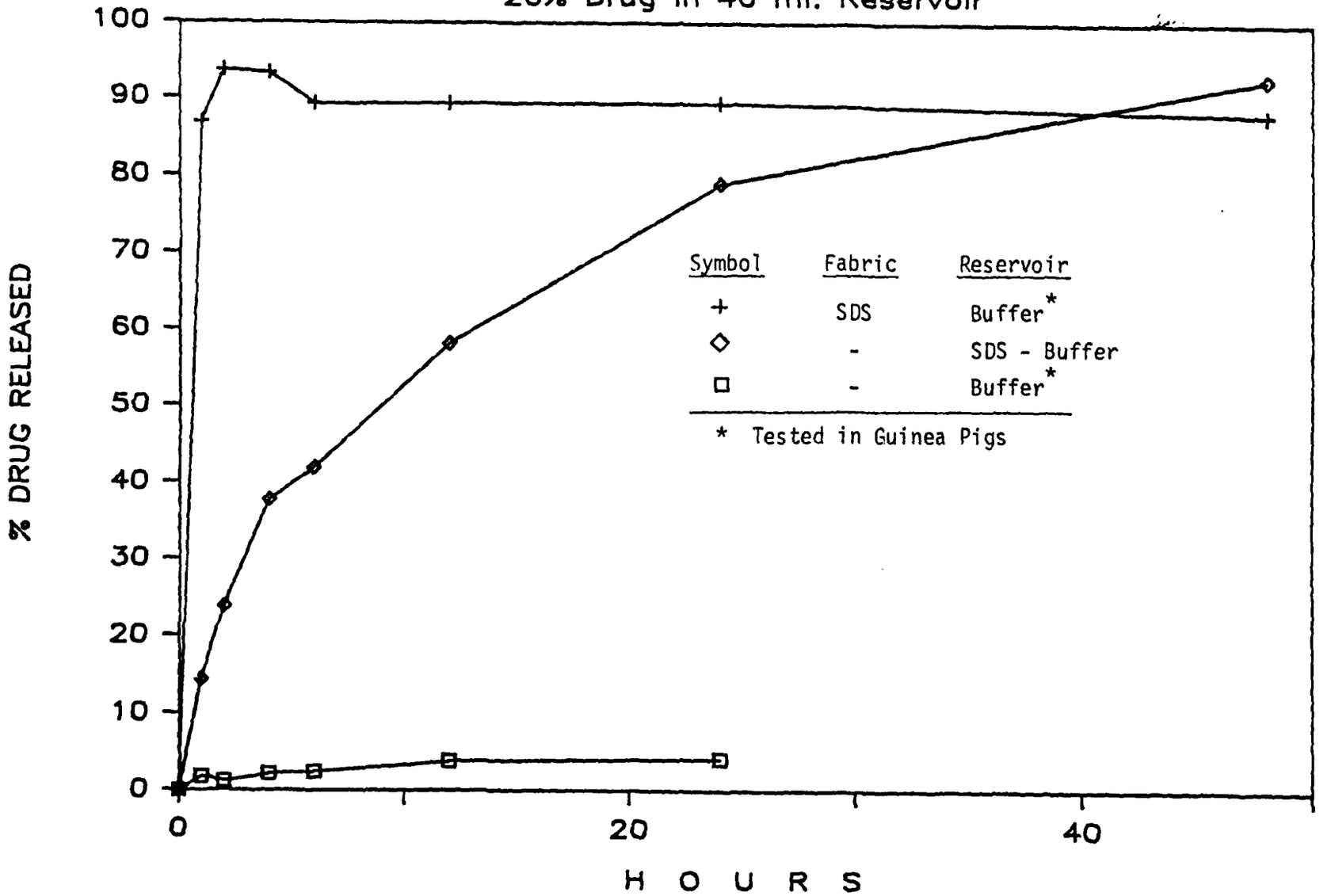
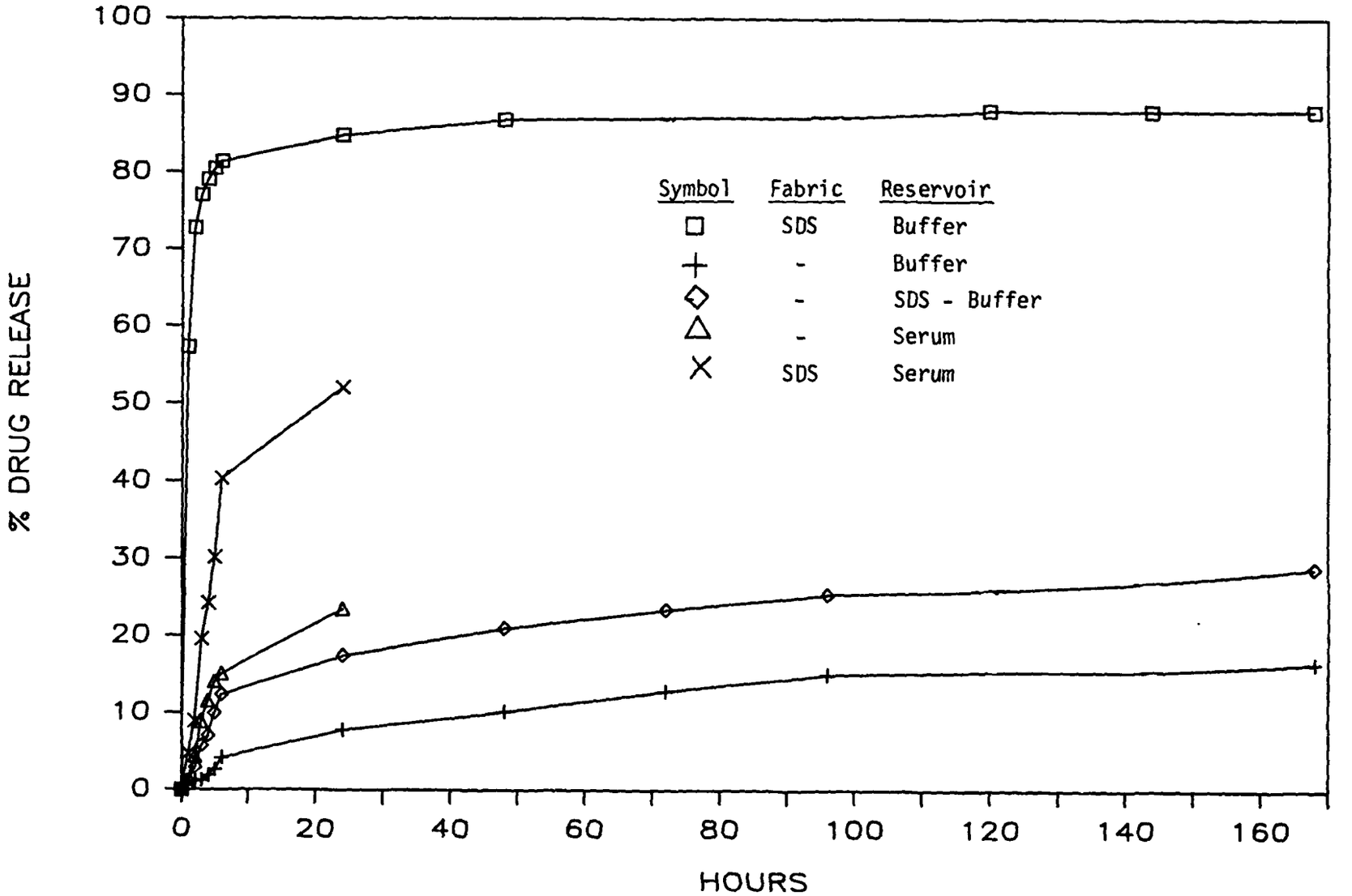


FIGURE 9

RELEASE FROM 20% AMPICILLIN TRIHYDRATE FABRICS
INTO 1 ml RESERVOIRS



serum was allowed to stand for 15 minutes prior to centrifuging. During the development of this procedure, the deproteinizing conditions were found to be important and a second peak was found for anhydrous ampicillin which was not present in the other ampicillin samples. A second peak also occurs in serum samples which have been in contact with ampicillin for several days. This is undoubtedly due to antibiotic decomposition. Solutions of pure drug can be followed in separate experiments to determine the rate of ampicillin (and clindamycin) decomposition in buffer and serum. The effect of decomposition on the uv assay of in vitro drug release can then be determined.

After the HPLC method was developed, fabric of ampicillin trihydrate (20%) was studied in the small reservoir cell using 1 ml of rabbit serum. Samples were taken at one hour intervals and after one day. These were analyzed using the HPLC conditions of Table 4. For the fabric without SDS the drug release (1% in one hour and 24% in one day) was faster than into buffer, but slower than into 0.001% SDS. For the fabric containing 1% SDS the drug release (5% in one hour and 52% in one day) was faster than without SDS in the fabric, but it was slower than the release of drug from this SDS fabric into buffer. Fabrics of 20% ampicillin trihydrate, with and without 1% SDS, have been studied at USAIDR in the guinea pig wound which is inoculated with S. aureus. A series of SEMs were taken by Colonel Mader at USAIDR and one of these is shown in Figure 10.

b) Anhydrous Ampicillin Fabrics

The measure of ampicillin release from the anhydrous ampicillin fabric was measured at 240 nm without regard to the presence of the impurity found by the HPLC method. Only a 20% anhydrous ampicillin fabric was prepared.

In the standard 40 ml cell the drug release into buffer was slow but significant (5% in one hour and 15% in one day). It was increased by addition of 0.001% SDS to the buffer (13% in one hour and 76% in one day). This data was similar to that for ampicillin trihydrate.

TABLE 4

CONDITIONS FOR AMPICILLIN ASSAY OF SERUM BY HPLC

Instrument Detector-Wavelength = 214 nm
-Flow Rate = 0.6 ml/min.

Mobile Phase - Methanol/Buffer = 30/70
Buffer (Phosphate) = pH 7, .017M

Sample - Protein Free Filtrate = 25µl

Column - Radial-Pak, µ-Bondapak, C-18 (5 µm, 8 mm x 10 cm)

Retention Time - 8 Minutes

FIGURE 10

SEM of 20% AMPICILLIN TRIHYDRATE NON-WOVEN FABRIC

In the small 1 ml cell the drug release was slower than in the agitated large volume cell at 37°C. In the small cell the fabric released 2% into buffer in one hour and 12% in one day. With SDS in the buffer the rate of release was still slow (1% in one hour and 14% in one day).

c) Sodium Ampicillin Fabrics

Sodium ampicillin did not form a fluffy non-woven fabric. The powdery material released 62% of the ampicillin in one hour and 74% in one day in the standard 40 ml cell using buffer. In the 1-ml cell the release was almost as fast (42% in one hour and 67% in one day). Measurements were not made in the presence of SDS.

2) Ampicillin Powders

For the first series of powders, films were prepared with 20% ampicillin trihydrate and sodium ampicillin. These films were cut and the pieces fed through the hammer mill. However, in this experiment dry ice was not mixed with the film and passed through the grinder as in the previous experiments. This avoided the build up of moisture on the product due to moisture condensation as the dry ice sublimed. The absence of dry ice did not appear to affect the product yield or the size distribution of the powder. This data is shown in Table 5.

A second series of powders were prepared with 5% and 20% anhydrous ampicillin, and 5% of sodium ampicillin, and ampicillin trihydrate. These powders were prepared with dry ice, but the product was quickly transferred to a vacuum oven and the dry ice sublimed into a vacuum line. A dry powder was obtained with yields as described in Table 5. Based on the problems of ampicillin decomposition, the latter preparation method is preferred.

Because of the fast release from the 20% ampicillin powders, at three chosen sieve ranges, powders were prepared at 5% of each of these drugs. The drug release was studied in the standard (40 ml) cells with buffer. Data was taken at several times and for several days. However, a summary of the data, as shown in Table 6, is sufficient to show the trend. In all cases drug

TABLE 5

SIZE DISTRIBUTION OF AMPICILLIN POWDERS

(Data is % of Total Milled Sample)

Size (μm)	Ampicillin Trihydrate		Ampicillin Sodium		Ampicillin Anhydrous	
	5%	20%	5%	20%	5%	20%
> 600	3.0	2.9	9.8	6.8	3.2	9.1
600-425	19.7	20.6	19.5	15.8	15.3	15.1
425-300	39.1	44.7	28.1	25.2	37.8	23.8
300-212	19.6	22.4	21.1	22.8	23.5	22.7
212-150	7.9	7.4	11.4	15.0	7.4	11.8
150-106	5.6	2.4	7.0	9.8	7.1	10.8
106-74	2.8	0.6	2.2	4.0	3.5	4.4
74-35	1.0	0.1	0.7	2.0	1.8	2.0
< 38	0	0	0.1	0	0.2	0.3
Yield	85	57	94	80	89	90

TABLE 6

CUMULATIVE % DRUG RELEASE FROM AMPICILLIN POWDERS INTO 40 ml RESERVOIRS

Sample	% Drug	Hours	Particle Size (μm) 75-106	212-300	425-600
Ampicillin sodium	20	1	88	84	78
		6	88	89	83
		24	84	92	94
	5	1	122	105	95
		6	127	111	109
		24	123	108	111
		48	115	102	105
Ampicillin trihydrate	20	1	85	75	79
		6	92	82	89
		24	86	81	85
	5	1	71	34	18
		6	79	39	23
		24	86	42	24
		48	84	42	23
Ampicillin anhydrous	20	1	89	84	60
		6	88	90	71
		24	84	85	71
		6 day	64	64	56
	5	1	82	54	36
		6	80	55	38
		24	71	47	29
		6 day	51	40	25

decomposition occurs concomitantly with drug rel[illegible] see no promising materials in this test, although drug release in a [illegible] and in vivo might be slower. Lower drug loading did not significa[illegible]ecrease the rate of ampicillin release. Microencapsulation appears to [illegible] a better alternative for the preparation of ampicillin particles.

3) Ampicillin Microcapsules

Recently microcapsules were prepared using sodium ampicillin and ampicillin trihydrate. The first encapsulation used a nondescript core of sodium ampicillin and should be repeated. This was the first drug encapsulation using the small air suspension coater. The second encapsulation used ampicillin trihydrate (H. Reisman) in the 2-inch coating chamber. Approximately 14 grams of core material was coated to 35% polymer wall with a 2% polylactide solution. Small samples were taken at 6, 8, and 15% coating.

Drug release studies were performed on unsieved material of the smallest coating samples (6 and 15% coated). At 8 and 35% coating, sieve fractions of 210-300 um were used for the drug release experiment. The data are shown in Figure 11. Data at more than 6 hours was compromised by drug decomposition. However in this preliminary study the slow release of ampicillin was demonstrated, and the effect of higher coating levels giving slower drug release is evident.

4) Complex Ampicillin-Polymer Matrices

A comparison of the in vitro release rates of the three formulations of ampicillin trihydrate is shown in Figure 12. From this data it appeared that better control of release rate could be achieved by combining non-woven fabrics with either powders or microcapsules. A preliminary experiment was conducted to test the hypothesis that powders and microcapsules could be incorporated into the fabric. Microcapsules 100 - 600 microns with 65% ampicillin trihydrate or ampicillin powders 80% drug 100 -600 microns were suspended in a Wurster air suspension coating chamber. The coating nozzle was replaced with a

FIGURE 11

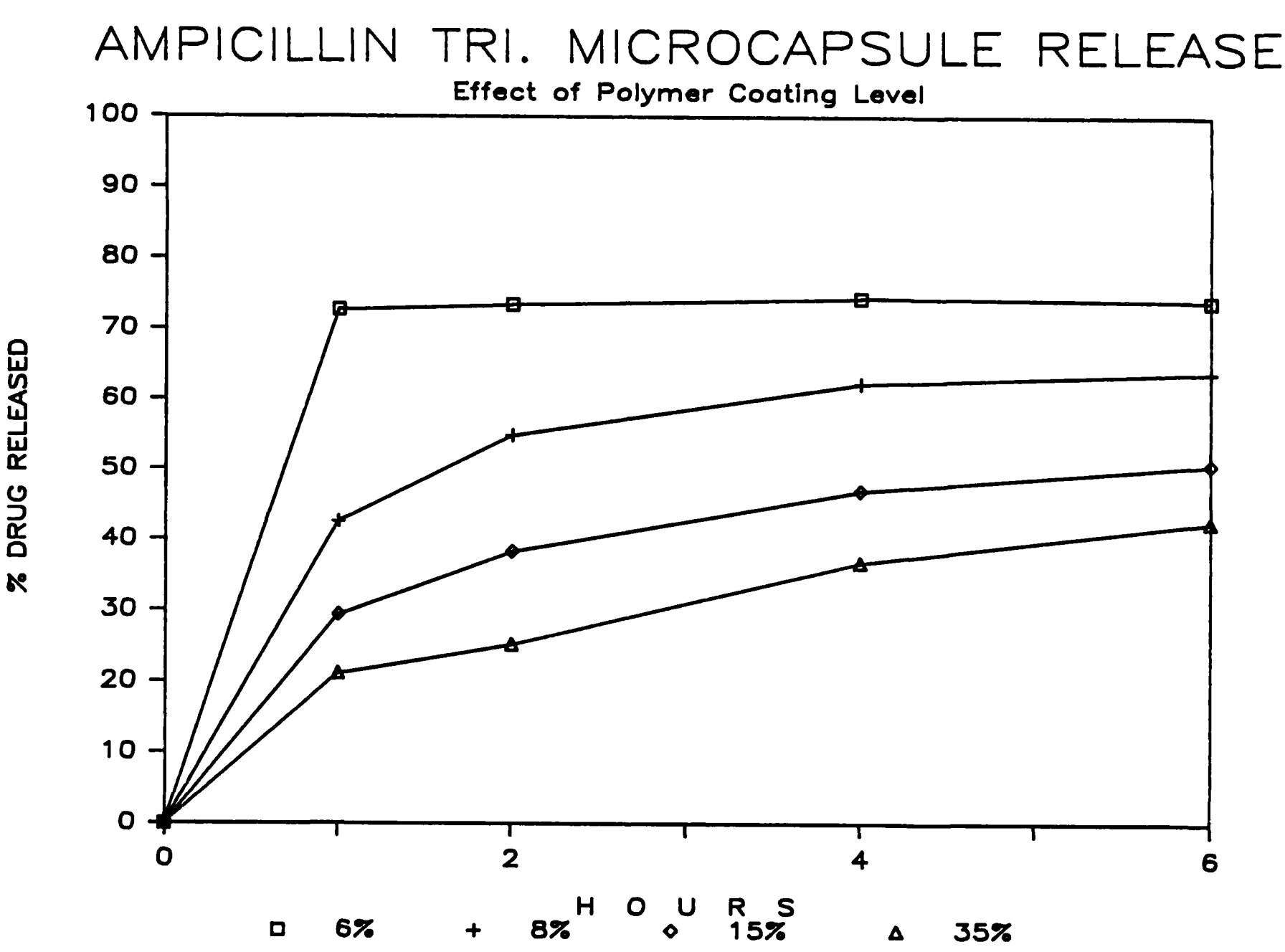
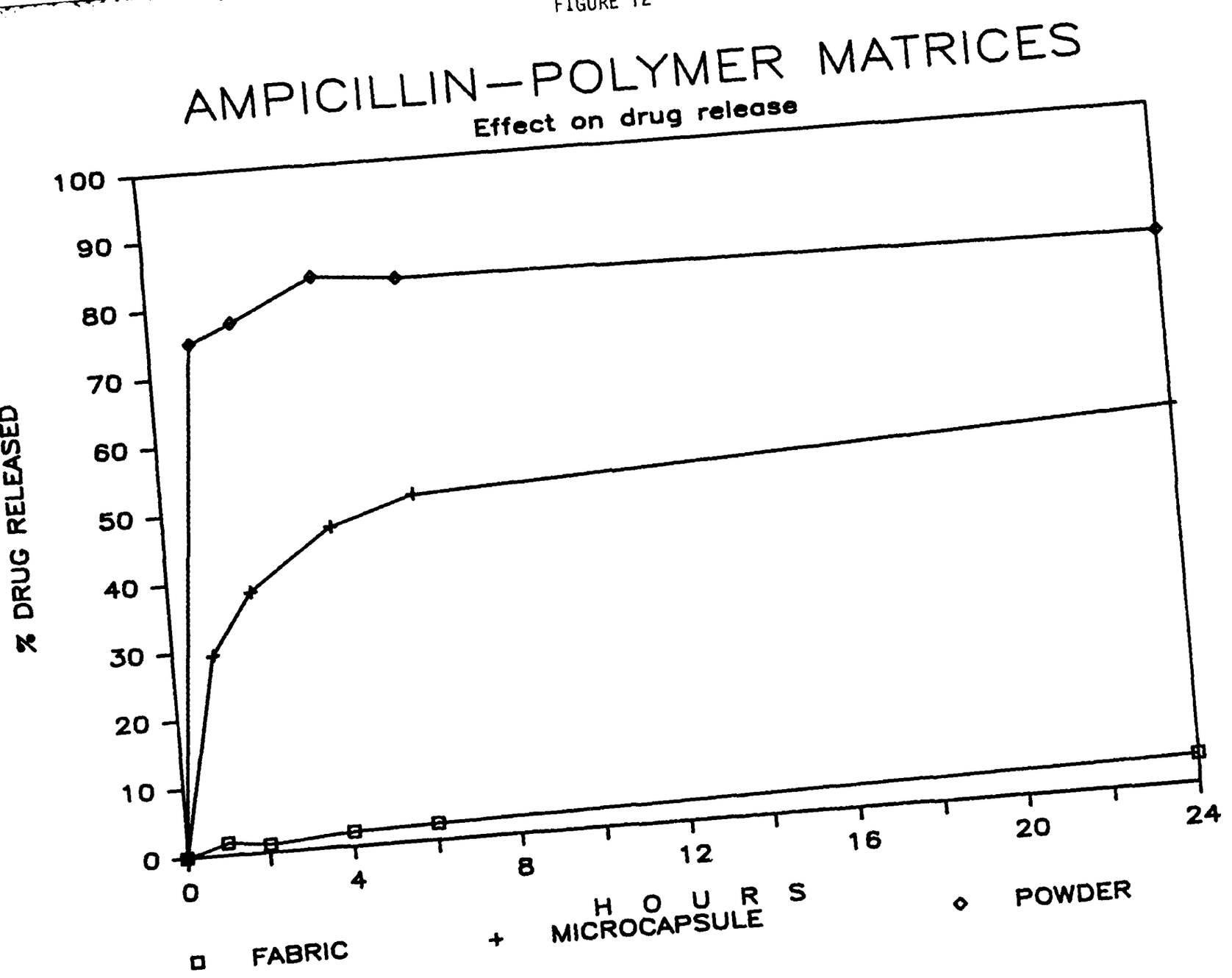

AMPICILLIN TRI. MICROCAPSULE RELEASE
Effect of Polymer Coating Level
% DRUG RELEASED
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
HOURS
6%
8%
15%
35%

FIGURE 12


AMPICILLIN–POLYMER MATRICES
Effect on drug release
% DRUG RELEASED
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
H O U R S
FABRIC
MICROCAPSULE
POWDER

fabric preparation nozzle and 10% polylactide in methylene chloride was blown through the suspended particles. As the fabric strands dried they trapped the particles and the resultant fabric was collected on a gauze backing.

Drug release from these complex matrices is shown in Figure 13. Pure drug and the powder both released almost instantly (< 15 minutes), and microcapsules released much slower. Powder release was significantly slowed by incroporation into the fabric. On the other hand the microcapsules incorporated into polymer fabric release ampicillin somewhat more rapidly than when tested above indicating that some of the microcapsule wall was removed during incorporation into the fabric.

This study indicates that it is feasible to prepare fabrics which incorporate microcapsules with desired release characteristics.

5) Ampicillin Stability

As previously noted ampicillin is not stable in solution, and in fact, an impurity was detected in the material supplied to us by Bristol Laboratories. Conversations with Mr. George Bittner of Bristol Laboratories confirmed this problem, but unfortunately he could offer no simple solutions. Based on their experience, we studied the stability of the drug in a number of solvent systems. Approximately 5 mg of ampicillin trihydrate was dissolved in 20 ml of solvent and at appropriate intervals 300 ul samples were mixed with 2.4 ml of methanol. Serum samples were then centrifuged to remove precipitated protein. The extract was then analyzed by H.P.L.C. and diluted 1/5 (v/v) with methanol and analyzed by direct u.v. absorbance. For both direct u.v. and H.P.L.C. the detection wavelength was 214 nm. Samples assayed 15 minutes after preparation of the solution were considered as 100% recovery. Appropriate blanks were included.

The data is shown in Table 7. The H.P.L.C. assay clearly shows that there is significant degradation of ampicillin in all solutions. In all conditions except calf serum, there is adequate stability for approximately 24 hours, but after 24 hours major losses occurred in all media except perhaps 40 mM phosphate buffer pH 6.5 which gave 88% recovery at 96 hours. The degradation

FIGURE 13

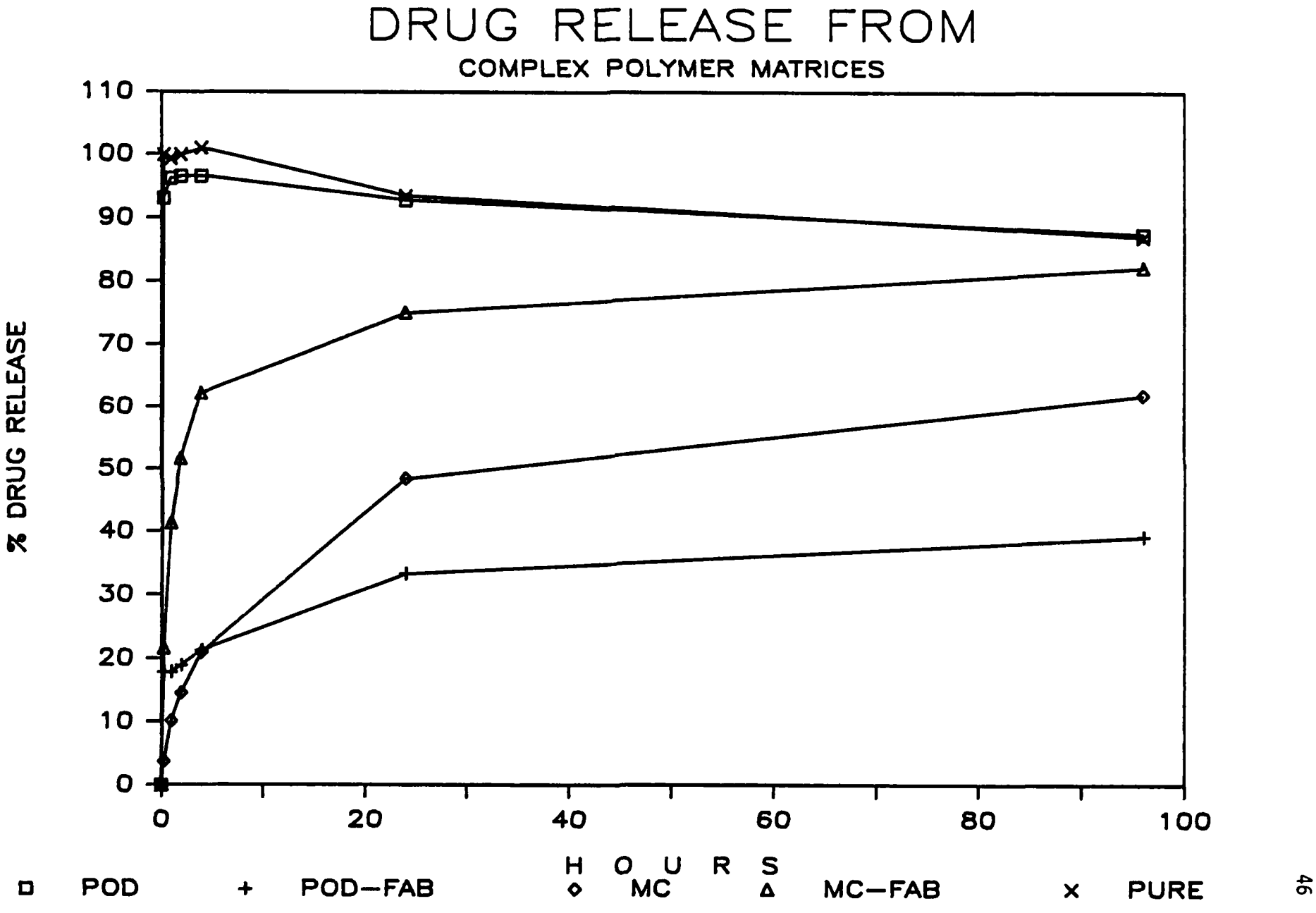

DRUG RELEASE FROM
COMPLEX POLYMER MATRICES
% DRUG RELEASE
110
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
H O U R S
POD
POD—FAB
MC
MC—FAB
PURE

TABLE 7

STABILITY OF AMPICILLIN

Medium	Time (hours)	% Recovery 1	2	4	6	24	48	96
		H.P.L.C. Assay						
Phosphate Buffer pH 7.4		102	108	110	101	95	49	-
pH 7.4 .001% SDS		98	92	-	91	86	65	-
pH 6.5		100	106	101	99	90	-	88
Calf Serum		109	100	107	96	37	0	-
Rabbit Serum		100	-	97	-	95	84	60
		U.V. Assay						
Phosphate Buffer pH 7.4		99	100	101	100	95	92	-
pH 7.4 .001% SDS		100	102	97	100	94	-	147
pH 6.5		106	100	100	90	90	-	100
Calf Serum		162	116	85	116	37	5	-

in the S.D.S. containing buffer is interesting since S.D.S. increases the solubility of ampicillin (See Section III.C.2.a.6). Comparisons of the H.P.L.C. and u.v. assays done on the same samples show that with ampicillin direct u.v. assay can lead to spurios results. Ampicillin degradation apparently leads to products which also absorb in the u.v. region and significant increases in apparent recovery were observed in calf serum and in the 96 hour sample in the S.D.S. containing buffer.

These observations suggest that particularly when direct u.v. absorbance assays are used the diffusion media should be replaced at least every 24 hours if not more often.

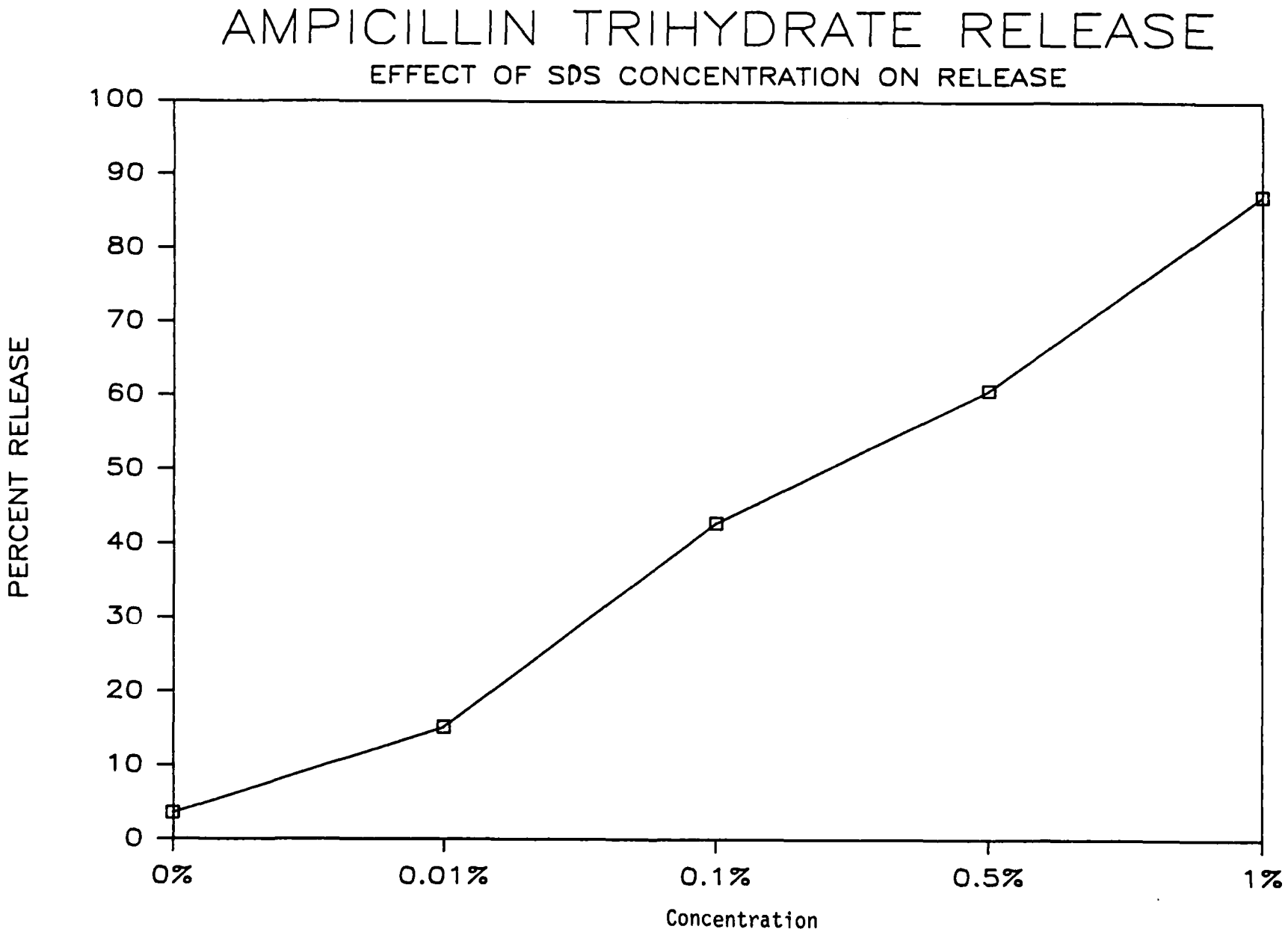
In order to assay antibiotics in the drug-polymer matrices it is necessary to either find a solvent in which both are soluble or develop an extraction procedure. While the polymer is soluble in dioxane, chloroform, and methylene chloride, ampicillin is insoluble in these materials. Since dioxane is a carcinogen it was not studied. The addition of methanol to methylene chloride does allow solution of ampicillin. Greater solubility is achieved when either excess sodium hydroxide or a 1:1 molar ratio of sodium hydroxide to ampicillin is also included. However, in these solutions 40% or greater degradation occurred in 30 to 60 minutes. In methanol or water with a 1:1 molar ratio of sodium hydroxide and ampicillin only 12% of the drug was lost in one hour.

An extraction procedure based on partition of polymer into chloroform and ampicillin into water was developed. Approximately 50 ng of matrix is dissolved in 10 ml of chloroform with mixing. Water, 10 ml, is added with vigorous mixing to dissolve the drug. With this system the mean recovery was 104 $\pm$ 0.9% (S.E.M.) for five samples.

6) Effects of S.D.S.

In all of the previous studies when sodium dodecylsulfate, S.D.S., is added either to a drug-polymer matrix or to the diffusion media, the rate of drug release increased dramatically. Figure 14 demonstrates the effect of increased S.D.S. concentration in the polymer matrix on release. These data may be explained in part by either an increase in fabric wettability or an increase in

FIGURE 14


AMPICILLIN TRIHYDRATE RELEASE
EFFECT OF SDS CONCENTRATION ON RELEASE
PERCENT RELEASE
100
90
80
70
60
50
40
30
20
10
0
0%
0.01%
0.1%
0.5%
1%
Concentration

drug solubility. To test the latter hypothesis, 60 mg of ampicillin trihydrate was suspended in 2 ml of distilled water with various concentrations of S.D.S. mixed for 15 minutes, and the excess separated by centrifugation. The amount of drug in solution was determined by u.v. absorption at 240 nm. The results, shown in Table 8, demonstrate a significant increase in drug solubility in the presence of S.D.S. S.D.S. appears to be an excellent material for regulating the release of ampicillin from a polymer matrix.

b. Clindamycin Materials

Clindamycin was the first material to be received and was the most difficult to analyze. Clindamycin has many structural groups which could be used as the basis of an analysis (thio, hexose, pyrrolidine, and amide groups). It was also expected to be a useful uv absorber. Unfortunately, clindamycin in acid or base did not absorb above 240 nm.

Attempts were made to develop a colorimetric assay using potassium permanganate (Draeger tube reactant for organic sulfides), an iodometric assay (USP assay for several antibiotics, including ampicillin), a hydroxylamine ferrous ammonium sulfate colorimetric assay (also USP for antibiotics), and a gas chromatographic procedure (USP for clindamycin). None of these methods were both simple and sensitive. An HPLC method was noted which used an uv detector at 214 nm; therefore we explored the lower uv range. In phosphate buffer, using a quartz cell, a direct uv reading at 220 nm was sufficiently sensitive to be used for in vitro drug release studies. At 220 nm the sensitivity of clindamycin in buffer is 1420 ug/ml-AU. Addition of NaOH increased the sensitivity to 806 ug/ml-AU, but the complication of diluting the sample, possible decomposition, and correcting for the discarded material did not warrant the use of added base.

The gas chromatographic method described in the USP included a derivitization step with acetic anhydride. This method was tested and could be used as an alternative. An HPLC method is also available (Landis, Grant, and Nelsen, 1980). A microbiological method was submitted to us by Dr. Zurenko at Upjohn.

TABLE 8

EFFECT OF S.D.S. ON AMPICILLIN SOLUBILITY

S.D.S. Concentration (%)	Ampicillin Solubility (mg/ml)
0.000	6.31
0.001	6.30
0.01	6.21
0.1	6.22
1.0	8.98
10.0	> 20.47

Clindamycin is available only as the hydrochloride and as clindamycin esters. The base form of clindamycin was prepared at BIOTEK by the neutralization of a clindamycin-HCl aqueous solution. The base was readily precipitated in water, filtered, and washed. Less soluble salts could also be easily prepared by precipitation with other anions (e.g., sulfate and phosphate, as we did with lidocaine).

1) Clindamycin Fabric Studies

Clindamycin-HCl does not codissolve with the polymer in methylene chloride solutions. However an excellent suspension is formed in methylene chloride which can be sprayed through an atomizing nozzle. A 20% drug fabric was prepared using our standard conditions.

Using 20% clindamycin-HCl fabric in the standard cell almost all of the drug was quickly released into the standard phosphate buffer (70% in one hour and 80% in one day). Fabrics were, therefore, prepared with 15, 10, and 5% drug. However, the release was not significantly slower at these higher polymer concentrations. There would be no apparent advantage to testing these fabrics in the presence of SDS. Clindamycin was released slower into the non-agitated 1 ml of buffer. The 20% clindamycin fabric released 33% of the drug in one hour and 46% in one day in the 1-ml test system.

The reproducibility of drug release from fabrics is much less than for similar powders or microcapule materials. Also for clindamycin we have not yet developed an assay procedure for the fabric. Hence the data is based on the weight of drug added to the polymer solution for the preparation of the fabric. However we believe that the non-reproducibility is due to the drug release test method, possibly a non-reproducible wetting of the test fabric in the test cell.

2) Clindamycin Powder Studies

Powders were prepared at 20% and 5% clindamycin-HCl. The drug release was studied in the standard (40 ml) cells with buffer. Data was taken at several times and for several days. However, a summary of the data, as shown in Table

9 , is sufficient to show the trend. For clindamycin, we suspect that there was drug segregation and that the small powder contains most of the drug. In all cases drug decomposition occurs concomitantly with drug release. However, we see no promising materials in this test. Powders of clindamycin base or less soluble salts, or clindamycin-HCl microencapsulation appear to offer better alternatives for clindamycin particulates.

3) Clindamycin Microcapsule Studies

Core particles of clindamycin hydrochloride were coated using the 2-inch coating chamber. Approximately 15 grams of material was coated to a level of 20%. A small sample was also taken at the 10% coating level.

These materials have not been tested for drug release at this time.

D. Stability of Stored Materials

The stability of the drugs in wound dressings under military field conditions needs to be addressed. Therefore preliminary experiments were performed to determine the stability of the drug (by *in vitro* assay) and the stability of drug release rate (also *in vitro*) as a function of various storage conditions. Relatively dry conditions of storage are required in order to maintain the drug within the polymer matrix. The poly-L(-)lactide is expected to be stable, but is hydrolyzed slowly in the presence of moisture. This is the basis of the biodegradation of the polymer to lactic acid in the body. As shown by the slow release of levonorgestrel from similar microcapsules (Contract No. NO1-HD-0-2847) this *in vitro* and *in vivo* degradation requires several months to achieve a significant breakdown of a poly-L(-)lactide (R.S.V. approximately 1 dl/g) microcapsule wall.

Six storage conditions were chosen (Table 10) which covered a range of temperatures, with and without laboratory levels of humidity and light. Two separate containers of each drug were prepared. One container was to be opened at the end of the first contract year for assay and release testing. The other container was to be used for two later analyses. American Can Company graciously supplied sufficient retortable pouches (M-1173 61-0.93004 heat-

TABLE 9

CUMULATIVE % DRUG RELEASE FROM CLINDAMYCIN POWDERS INTO 40 ml RESERVOIRS

Sample	% Drug	Hours	75-106	212-300	425-600
Clindamycin-HCl	20	1	133	62	40
		6	135	64	48
		24	142	65	51
	5	1	82	49	41
		6	79	43	41
		24	51	18	14
		6 day	37	5	6

TABLE 10

SAMPLE STORAGE CONDITIONS

CONDITION	TEMPERATURE	HUMIDITY	LIGHT
1	40°C	Ambient	None
2	40°C	Desiccate	None
3	Ambient	Ambient	None
4	Ambient	Ambient	Ambient
5	4°C	Ambient	None
6	4°C	Desiccate	None

sealable bags) for dessicant storage. Ambient light and moisture was achieved in glass vials capped with glass wool. Absence of light was achieved with black tape around the vials. Temperatures were 40°C (chemical oven), ambient (laboratory), and 4°C (refrigerator). In the analysis of the data it must be remembered that different people, using slightly different techniques took the data in different years. Hence only gross changes may be significant. The samples which have been stored are listed in Table 11. Two early samples of povidone iodine fabric and powder were stored, but were discarded after being replaced by more appropriate samples. All of the anesthetic samples have now been removed from the testing protocol.

1. Storage of Anesthetic Wound Dressings

The release of any drug from a fabric matrix was less reproducible than from powders or microcapsules, presumably because of non-reproducible wetting of the fabrics. Benzocaine fabrics may be considered to be relatively stable, although some increase in the initial release may occur after storage at 40°C Etidocaine-HCl in fabric form is stable. Lidocaine base is not apparently lost from the system (fabric assays are stable). However, after storage at higher temperatures, the drug is released from the fabric more rapidly.

The anesthetic release from powders was more reproducible, but perhaps less significant than the study of the anesthetic microcapsules (Contract DAMD17-81-C-1195). Benzocaine was lost from a 20% powder when stored, unsealed, at 40°C. There was also some redistribution of benzocaine in the sealed container at 40°C. This led to a burst of benzocaine release from these samples. Etidocaine-HCl powders were stable under all conditions. Lidocaine-HCl in 20% powder did not have significant slow release properties. The assay of the powders after storage was consistent with no decomposition of the material. Lidocaine base is a low melting compound which appeared to redistribute at all temperatures, but more rapidly at high temperatures. However the total lidocaine in the powder was constant in these powders.

TABLE 11

SAMPLES STORED FOR STABILITY TESTS

Fabric Samples

Drug	% Drug	Reference	Date Stored
Benzocaine	20	8/44	7/15/81
Etidocaine-HCl	20	8/44	7/15/81
Lidocaine Base	20	8/44	7/15/81
PVP-I_2	40	44/85	6/2/82
Benzalkonium Chloride	20	-	3/7/83
Ampicillin Trihydrate	20	-	--

Powder Samples

Drug	% Drug	Size (μm)	Reference	Date Stored
Benzocaine	20	212-300	8/34	4/29/81
Etidocaine-HCl	20	212-300	8/34	4/29/81
Lidocaine-HCl	20	212-300	8/34	4/29/81
PVP-I_2	20	300-425	8/44	7/15/81
Lidocaine Base	10	300-425	44/85	6/2/82
Benzalkonium Chloride	20	425-600	-	3/7/83
Nitrofurazone	40	300-425	-	--

Microcapsules Samples

Drug	% Drug	Size (μm)	Reference	Date Stored
PVP-I_2	49	212-600	25/78	3/7/83

2. Storage of Antiseptic Wound Dressings

Fabrics and powders of povidone iodine did not demonstrate significant sustained release. However the materials were stored to assess the stability of BASF 17/12 povidone iodine under various storage conditions. The povidone iodine was stable under these test conditions (Tables 12 and 13).

E. Biological Testing

1. Anesthetic Effectiveness

We had initially proposed to evaluate anesthetic preparations in guinea pigs by measuring the response to a tactile stimulus. However, our consultants from Astra Pharmaceuticals suggested that the change in the normal stance of a rat due to blockage of the sciatic nerve is a more reproducible method of determining local anesthetic effectiveness. Most of this work has been performed on a parallel contract (DAMD17-81-C-1195).

In order to use this approach with fabrics or large powders, it would be necessary to cut through skin and implant the material near the sciatic nerve. These materials cannot be injected through a hypodermic needle. Preliminary experiments were therefore performed to develop this method.

In the first experiment we used 45 mg/kg of ketamine plus 5 mg/kg of xylazine intramuscularly as the general anesthetic in 400 gram rats. We rolled a 1 cm x 1 cm piece of fabric into a 1 cm long x 0.3 cm diameter pledget. The rat thigh was shaved and scrubbed, and a 2 cm incision was made parallel to the femor. The muscle layer was dissected until the pledget could be inserted adjacent and parallel to the femur. The incision was then closed with wound clips through the skin.

Control experiments were performed in which polymer fabric (negative) and etidocaine microcapsules (positive) were implanted by a similar procedure. A sciatic nerve block could not be differentiated from general anesthesia for the

TABLE 12

STABILITY OF STORED ANTISEPTIC FABRICS

(Percent of Assay Value Released at Specific Times)

Drug	Years Stored	Hours of Release	Original Data	Storage Conditions: 40°C Dark Unsealed	40°C Dark Sealed	R.T. Light Unsealed	R.T. Dark Unsealed	4 °C Dark Unsealed	4 °C Dark Sealed
PVP-I_2 40%	1	1	72	85		78	75		
		6	100	95		89	91		
		24	119	100		97	96		
		Assay	(40)	38		38	38		

TABLE 13

STABILITY OF STORED ANTISEPTIC POWDERS

(Percent of Assay Value Released at Specific Times)

Drug	Years Stored	Hours of Release	Original Data	Storage Conditions: 40°C Dark Unsealed	40°C Dark Sealed	R.T. Light Unsealed	R.T. Dark Unsealed	4 °C Dark Unsealed	4 °C Dark Sealed
PVP-I_2 20% 300-425 µm	1	1	72	93		76	78		
		6	79	93		76	78		
		24		93		76	78		
		Assay	(20)	15		16	17		
	2	1	72	80		90	97		
		6	79	80		90	90		
		24		82		95	93		
		Assay	(20)	15		15	15		

first two to three hours, and no nerve block was observed with the etidocaine microcapsules or with the fabric control. Some anesthesia was observed in the rat with etidocaine fabric but this wore off within 12 hours.

In a second experiment rats were anesthetized with 40 mg/kg of sodium pentobarbital, administered intraperitoneally. In this case a 3 cm incision was made and the muscles were divided by blunt dissection. The sciatic nerve was found and gently freed from the surrounding tissue with forceps. The anesthetic preparation was inserted and the area wetted with 0.25 ml of sterile saline.

In this experiment 20 mg of etidocaine as pure drug crystals, 40 mg in microcapsules, and 7 mg and no drug in fabric (1 x 2 cm) was used. The animals recovered in 1 to 2 hours and nerve blocks were observed. The pure drug was effective for about 6 hours and the etidocaine fabric gave an anesthetic response for about one hour. There was no nerve blockage due to implanting the pure polymer fabric. The rat with implanted microcapsules gave variable responses. A larger pledget of fabric should be used to deliver more drug.

The testing of anethetics by this method was successfully used on Contract DAMD17-81-C-1195, with etidocaine microcapsules producing nerve blocks which lasted for 48 hours. However, the testing of anesthetics was deleted from the work scope of this contract at this point.

2. Antimicrobial Testing at USAIDR

The microbiological testing of the antiseptic and antibiotic wound dressings has been performed by the contracting organization (U.S. Army Institute of Dental Research) under the direction of Colonel Jack W. Vincent. Both *in vitro* microbial sensitivity and *in vivo* wound sepsis and healing tests are being performed by the Army personnel.

Initially a test protocol was written at USAIDR (Vincent, Setterstrom, Hollinger, 2 November 1982, "*In Vivo* Evaluation of a Wound Dressing Containing Poly-L(-)lactide and Povidone Iodine"). This included both the effect of wound dressings on antiseptic efficacy and the effect on wound healing.

a. Preliminary Study

Based on this protocol we chose the following non-woven fabric materials for the initial in vivo studies (Table 14). Strips of non-woven fabric which were at least 1.5 cm wide and about 8 cm long were sent to Dr. Vincent. The backing was Parke-Davis Gauze bandage. The strips can be cut with surgical scissors to give the correct dimensions for each wound. In order to achieve continuous release of a small quantity of BAC, a 20% BAC/polymer solution was sprayed on top of a polymer fabric. In vitro studies indicate a continued release of similar samples. The samples were handled with surgical gloves and heat sealed in plastic envelopes prior to shipment.

In this first study, ten animals were used with each type of bandage for measurement of the antiseptic efficacy. The same technique had been used to evaluate another wound dressing generating reproducibly (100% morbidity) contaminated wounds (Vincent, et al, 1982, personal communication, test protocol). However, the BIOTEK fabric wound dressings were more absorbant and the control dressings provided contaminated wounds in only 3 of 10 cases (30%). In test animals, 0% of the povidone iodine dressings produced contaminated ...ds (0/9) and 10% of the BAC treated sites were contaminated (1/10). Plans were made to modify the procedure to insure infection at the control sites when using polylactide control dressings.

b. Povidone Iodine Studies

In the next set of experiments both povidone iodine and nitrofurazone were studied by microbiological in vitro and in vivo methods. By using povidone iodine on agar discs a minimum povidone iodine concentration was determined which was effective against the Staphylococcus aureus strains being used in the in vivo studies. A zone of inhibition was found at 15 ug/ml for both Strain 12600 and 6538P.

A guinea pig study was then initiated with a inoculum of 1.5 x 10 S. aureus 6538P microorganisms under a full thickness dissection flap. Four

TABLE 14

INITIAL SAMPLES SENT FOR IN VIVO
EVALUATION ON WOUND HEALING OF ARTIFICIALLY
CONTAMINATED WOUNDS

Sample	Thickness (mm)	Fabric Wt. mg/cm^2	Drug %	Drug Wt. mg/cm^2
Control	1.04±0.15	14.8	0	0
PVP-I_2	1.19±0.20	19.8	40	7.9
BAC	1.12±0.25	15.4	2.7	0.42

groups of 5 animals were used as shown in Table 15. Both the bandages and the wound areas were analysed for bacteria with a spiral plater. Scrubs were taken with 2 ml of detergent buffer, and bandages were placed in 1 ml of buffer.

The data were transformed to a logarithmic base for ease of analysis and for the appropriate student-t test. The only significant effect was the decrease of wound and bandage microorganisms when using the nonmedicated dressing with pure povidone iodine (Group 2 vs. 3 for wound $t = 7.4$ and for dressing $t = 2.8$). However the counts of 2×10^5 cfu/ml of Group 3 would still have to be considered "non-sterile". In these experiments the bandages were not pre-wet with saline. Thus the absorptive property of the bandage was used to our advantage, as in the preliminary experiment.

Povidone iodine powder was tested for antimicrobial efficacy in combination with the wound dressing (Table 16). For each test group five wounds (2 per guinea pig) were infected with 1.26×10^9 c.f.u. of S. aureus 12600. The addition of 50 ul of PVP-I_2 solution to the control fabric appears to reduce the bacterial count by approximately 1 log unit (Groups 2 and 3 versus 1). The addition of 7 mg of PVP-I_2 powder, however, had no significant effect (Group 4 versus 1). In none of the efficacy studies did PVP-I_2 successfully control the induced infection.

In a peritonitis model, S. aureus was used in preliminary experiments. The infection was too rampant to be controlled by antibiotics or antiseptics. Deaths occurred within hours. Using E. coli as the inoculum generated a more realistic infection (9 of 10 died in a day). This was controlled by an antibiotic preparation (0/10 died), but not with 24 mg of povidone iodine (9/10 died).

c. Nitrofurazone Studies

When a sample of nitrofurazone arrived at BIOTEK a sub-sample was sent to Dr. Vincent, along with a microbiological test method which we received from the supplier, Norwich-Eaton Pharmaceuticals. Using this method, minimum effective concentrations of nitrofurazone were determined against the two strains of S. aureus. The concentration of 6.2 ug/ml was effective against

Table 15

EFFECT OF POVIDONE IODINE PREPARATIONS
ON CONTAMINATED WOUND
Log (CFU/ml) as Mean ± S.D. (no. of animals)

	Group	Wound	Dressing
1	No Dressing	6.63±0.87 (5) 6.24±0.08 (4)	No dressing No dressing
2	Control Dressing	6.02±0.09 (4)	6.01±0.54 (4)
3	Control Dressing + 22 mg drug	5.32±0.17 (5)**	4.86±0.66 (5)***
4	PVP-I_2 Dressing Bandage* + 22 mg drug	5.96±0.46 (5)	6.09±0.29 (5)

* Drug in bandage is approximately 8 mg (8 mg/cm^2)

** $p < .001$

*** $p < .025$

TABLE 16

EFFECT OF POVIDONE IODINE POWDER

ON CONTAMINATED WOUNDS

Treatment	Wound	Dressing	Total
1. Control Dressing	6.000	6.673	6.757
2. Control Dressing + 50 µl PVP-I_2	5.531	4.883	5.619
3. Control Dressing + 50 µl PVP-I_2	5.756	5.653	6.009
4. PVP-I_2 Dressing* + 7 mg PVP-I_2 powder	5.477	6.734	6.757

Data are expressed as mean log C.F.U. n = 5 for all groups.
Data supplied by Dr. J.W. Vincent, USAIDR.

*PVP-I_2 dressing contains approximately 8 mg of PVP-I_2.

Strain 6538 and 12.5 ug/ml was required for Strain 12600. These results were based on the visible turbidity of solutions after one day at 37°C. Aliquots from tubes suggesting bactericidal effect were plated on tryp.-soy agar. Following incubation at 37°C for 24 hours each plate showed high colony counts indicating a bacteriostatic rather bactericidal effect.

Two guinea pigs were used with a 1.3×10^9 cfu S. aureus 12600 inoculum after exposing subcutaneous adipose tissue using the flap technique. In one guinea pig, 10 mg of pure nitrofurazone was used. The wound sample contained 1.4×10^5 cfu/wash. In the second animal 5 mg of nitrofurazone was used and the scrub contained about 1.6×10^6 cfu/wash. Thus more than 10 mg of nitrofurazone appears to be required to control this type of infection, when using this method of analysis.

In the next experiment 40% nitrofurazone powder (300-425 um), 25 mg (10 mg drug) was used in each of 15 guinea pigs. A separate group of 15 guinea pigs were used as controls (no powder or drug added). Each group of 15 was split into thirds and sacrificed at one, two, and three days after the inoculation and implantation of test material.

In the first group of animals (1 day after powder implantation) it appeared that about 50% of the nitrofurazone powder remained unchanged. This might indicate that less than the anticipated amount of drug had been released from the powder. The powder should turn from brown to white as the drug is released. Results at three days suggested the presence of unaltered nitrofurazone similar to day one.

The data of USAIDR was sent to BIOTEK and was transformed into a logarithmic form and analyzed by a Student t-test. This analysis is shown in Table 17 and 18. There is a statistically significant difference between the test and control groups. However only two wounds of the test group could be considered completely aseptic, and one of these should be considered questionable since hemorrhaging occurred which required removal of the clot. Five additional test wounds had bacterial counts of less than 10^5 cfu/wash (wash is 2 ml of detergent solution). Only one control wound was less than 10^5 cfu/wash.

TABLE 17

USAIDR Data On Nitrofurazone Powder
Implantation In Guinea Pigs

	cfu/ml		log (cfu/ml)		
	Control	Test	Control	Test	
Day 1	4.0×10^7	0 (1)	7.60	(0)	
	4.4×10^5	4.0×10^4	5.64	4.60	
	3.5×10^4	1.5×10^4	4.54	4.18	
	1.5×10^5	1.2×10^5	5.18	5.08	
	3.9×10^5	4.5×10^4	5.59	4.65	
Average	8.2×10^6	4.4×10^4	5.71	4.63	(4)
S.D.			±1.14	±0.37	
Day 2	1.4×10^7	0 (1)	7.15	(0)	
	8.6×10^6	2.8×10^6	6.93	6.45	
	3.7×10^6	7.7×10^5	6.57	5.89	
	3.8×10^6	2.0×10^6	6.58	6.30	
	4.8×10^6	2.7×10^4	6.68	4.43	
Average	7.0×10^6	1.1×10^6	6.78	5.77	
S.D.			±0.25	±0.92	(4)
Day 3	7.8×10^6	1.8×10^5	6.89	5.25	
	8.0×10^6	8.0×10^2	6.90	2.90	
	5.0×10^6	3.4×10^5	6.70	5.53	
	1.2×10^6	6.8×10^5	6.08	5.83	
	1.0×10^6	6.0×10^6	6.00	6.78	
Average	4.6×10^6	1.4×10^6	6.51	5.26	
S.D.			±0.44	±1.43	

TABLE 18

ANALYSIS OF TOTAL GROUP
(USAIDR Data)

	n	log (cfu/ml)	cfu/ml
Control	15	6.335 ± 0.819	2.16×10^{6}
Test	15	4.525 ± 2.089	3.35×10^{4}
	14	4.848 ± 1.736	7.05×10^{4}
	13	5.221 ± 1.074	1.66×10^{5}

$n_1 = 15$	$n_2 = 15$	$t = 3.126$	d.f. = 28	$p < .005$
$n_1 = 15$	$n_2 = 14$	$t = 2.986$	d.f. = 27	$p < .005$
$n_1 = 15$	$n_2 = 13$	$t = 3.112$	d.f. = 26	$p < .005$

This data is comparable to a preliminary test (one guinea pig) in which 10 mg of pure nitrofurazone gave a value of 1.4×10^5 cfu/wash (7×10^4 cfu/ml).

In summary, 10 mg of nitrofurazone in a slow release powder reduced the standardized bacterial contamination by about 10-fold. It would be difficult to supply more than 25 mg of powder to a $1cm^2$ wound area in an effective wound dressing. However more than 40% drug might be able to be incorporated into the test powder. Faster or slower releasing powders (in in vitro tests) might be more advantageous. It is also important to show an advantage relative to the pure nitrofurazone powder (immediately available drug). Finally, a 10-fold decrease in microbial contamination is rather inadequate when compared to antibiotic treatments. Therefore, the next study was performed with ampicillin as the active material.

d. Ampicillin Studies

Ampicillin and clindamycin were tested simultaneously against S. aureus 12600. Discs of 8mm (50 mm^2) were impregnated with 10 ul of ampicillin solutions. For trihydrate the inhibitory quantity was 0.04 ug (4 ug/ml) per 50 mm^2. For the anhydrous ampicillin, 0.07 ug (7 ug/ml) was required. In the tube dilution test the MIC was 1.5 ug/ml for the anhydrous ampicillin and 3 ug/ml for the trihydrate. Sodium ampicillin was not tested because a relative assay value was available from Bristol and the wound dressings containing this material have shown less promise.

Since ampicillin was shown to be very effective against S. aureus and a promising ampicillin trihydrate fabric has been prepared, pure ampicillin trihydrate was placed in the inoculated guinea pig wound. Quantities of 20, 10, and 1 mg of drug were placed in the 1 cm wounds. The wounds were not aseptic, although the 20 and 10 mg test areas had lower c.f.u. counts. The wound areas were washed after 3 days of exposure. The control wound had 2×10^8 cfu, the 1 mg wound had 1×10^7 cfu, the 10 mg wound had 8×10^6 cfu, and the 20 mg ampicillin wound had 3.5×10^6 cfu in the wash.

In the next experiment ampicillin trihydrate fabric (20%) was placed in the standardized guinea pig wound which was infected with 1.6×10^9 cfu of S. aureus 12600. Five control animals and five test animals were sacrificed on Days 1 and 2. There was a significant decrease in the colony counts at Day 2 and it was suggested that the remaining groups be sacrificed at six days. At this time four of the five wounds were essentially sterile (Table 19).

Based on this information the remaining animals of this lot were treated with the 20% ampicillin trihydrate and 1% SDS fabric. This should release ampicillin more rapidly into the area of the wound. Only two times could be chosen, which were 2 and 6 days. The data, shown also in Table 19, shows again a sterile wound at six days (5 of 5). However, again the wounds were not sterile at Day 2.

Approximate levels of ampicillin were measured at USAIDR using the microbiological test method and knowing the minimum inhibitory concentration for the microorganism. From this data (Table 20) it appears that ampicillin is rather rapidly delivered by the test fabrics. Also there is less ampicillin remaining in the fabric containing SDS than in the fabric without SDS.

e. Clindamycin and Chlorhexidine Diphosphanilate Studies

The Upjohn sample of clindamycin-HCl was tested at USAIDR against the S. aureus strain which was normally used for wound inoculation (ATCC 12600). After seeding Mueller-Hinton agar plates, all dilutions of clindamycin (1.0 - 0.002 mg/ml) showed a hazy area of growth, with two rings. This was indicative of some growth inhibition, but not of good antibiotic susceptibility. In tube dilution tests there was growth of the microorganism at all dilutions. Based on this information we should not proceed to test our wound dressings using this strain of S. aureus in guinea pigs. The result is surprising since clindamycin is known to be effective against 96% of the S. aureus strains.

The chlorhexidine diphosphanilate was not effective against S. aureus and a different bacteria will have to be used to test this drug.

TABLE 19

AMPICILLIN FABRIC IN GUINEA PIG WOUND

MICROBIAL COUNTS ***

Experiment 1 - 20% Ampicillin Trihydrate Fabric (1.6×10^9 cfu, t = 0)

	Mean cfu's		
	1 day	2 day	6 day
Control			
Wound	9.0×10^6	3.8×10^7	8.8×10^6
Bandage	3.0×10^6	1.3×10^8	1.6×10^7
Total	1.2×10^7 (5)	1.7×10^8 (5)	2.5×10^7 (5)
Experimental			
Wound	6.0×10^6	3.9×10^6	11
Bandage	5.6×10^6	6.5×10^5	4
Total	1.2×10^7 (5)	4.6×10^6 (3)**	15 (4)*

* One animal not sterile, 4.4×10^5 cfu wound 4.7×10^5 cfu bandage
** Two contaminated

Experiment 2 - 20% Ampicillin Trihydrate + 1% SDS Fabric (1.1×10^9 cfu)

Control			
Wound	-	3.8×10^6	1.7×10^6
Bandage	-	8.2×10^5	6.6×10^4
Total	-	4.7×10^6 (2)	1.7×10^6 (2)
Experimental			
Wound	-	1.6×10^6 (6)**	23
Bandage	-	3.1×10^5 (5)	14
Total	-	1.9×10^6 (5)	37 (5)*

* One contaminated
** One bandage almost sterile, not included

*** Data provided by Dr. Jack W. Vincent, USAIDR

TABLE 20

AMPICILLIN FABRIC IN GUINEA PIG WOUND

AMPICILLIN CONCENTRATIONS*

Experiment 1 - 20% Ampicillin Trihydrate Fabric (approximately 3.8 mg)

	Concentration of Ampicillin (μg/ml)		
	1 day	2 day	6 day
Wound (2 ml total)	12 ± 7	3.9 ± 2.1	1.7 ± 1.1
Bandage (2 ml total)	258 ± 106	82 ± 53	66 ± 42
Serum	0.12 ± 0.11	0	0

Experiment 2 - 20% Ampicillin Trihydrate + 1% SDS Fabric

Wound	-	3.2 ± 3.2	2.1 ± 2.1
Bandage	-	22 ± 19	9.7 ± 6.8
Serum	-	0	0

* Data provided by Dr. Jack W. Vincent, USAIDR

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